National Library
of Canada

Acquisitions and
Bibliographic Services Branch

395 Wellington Street
Ottawa, Ontario
K1A 0N4

Bibliothèque nationale
du Canada

Direction des acquisitions et
des services bibliographiques

395, rue Wellington
Ottawa (Ontario)
K1A 0N4

Your file Votre référence

Our file Notre référence

NOTICE

The quality of this microform is heavily dependent upon the quality of the original thesis submitted for microfilming. Every effort has been made to ensure the highest quality of reproduction possible.

If pages are missing, contact the university which granted the degree.

Some pages may have indistinct print especially if the original pages were typed with a poor typewriter ribbon or if the university sent us an inferior photocopy.

Reproduction in full or in part of this microform is governed by the Canadian Copyright Act, R.S.C. 1970, c. C-30, and subsequent amendments.

AVIS

La qualité de cette microforme dépend grandement de la qualité de la thèse soumise au microfilmage. Nous avons tout fait pour assurer une qualité supérieure de reproduction.

S'il manque des pages, veuillez communiquer avec l'université qui a conféré le grade.

La qualité d'impression de certaines pages peut laisser à désirer, surtout si les pages originales ont été dactylographiées à l'aide d'un ruban usé ou si l'université nous a fait parvenir une photocopie de qualité inférieure.

La reproduction, même partielle, de cette microforme est soumise à la Loi canadienne sur le droit d'auteur, SRC 1970, c. C-30, et ses amendements subséquents.

Canada

UNIVERSITY OF ALBERTA

BIOCHEMISTRY AND HISTOLOGY OF THE FETAL HUMAN TEMPOROMANDIBULAR JOINT ARTICULAR DISC

BY

JAMES MAH ©

A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfilment of the requirements for the degree of **Master of Science**.

IN

CLINICAL SCIENCES AND ORAL BIOLOGY

FACULTY OF DENTISTRY

EDMONTON, ALBERTA

FALL 1992

National Library of Canada | Bibliothèque nationale du Canada

Canadian Theses Service | Service des thèses canadiennes

Ottawa, Canada
K1A 0N4

The author has granted an irrevocable non-exclusive licence allowing the National Library of Canada to reproduce, loan, distribute or sell copies of his/her thesis by any means and in any form or format, making this thesis available to interested persons.

L'auteur a accordé une licence irrévocable et non exclusive permettant à la Bibliothèque nationale du Canada de reproduire, prêter, distribuer ou vendre des copies de sa thèse de quelque manière et sous quelque forme que ce soit pour mettre des exemplaires de cette thèse à la disposition des personnes intéressées.

The author retains ownership of the copyright in his/her thesis. Neither the thesis nor substantial extracts from it may be printed or otherwise reproduced without his/her permission.

L'auteur conserve la propriété du droit d'auteur qui protège sa thèse. Ni la thèse ni des extraits substantiels de celle-ci ne doivent être imprimés ou autrement reproduits sans son autorisation.

ISBN 0-315-77298-0

Canada

UNIVERSITY OF ALBERTA
RELEASE FORM

NAME OF AUTHOR: **JAMES MAH**

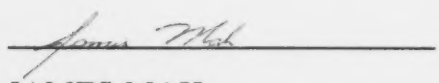
TITLE OF THESIS: **BIOCHEMISTRY AND HISTOLOGY OF THE FETAL HUMAN TEMPOROMANDIBULAR JOINT ARTICULAR DISC**

DEGREE: **MASTER OF SCIENCE**

YEAR THIS DEGREE GRANTED: **1992**

Permission is hereby granted to the University of Alberta Library to reproduce single copies of this thesis and to lend or sell such copies for private, scholarly, or scientific research purposes only.

The author reserves all other publication and other rights in association with the copyright in the thesis, and except as hereinbefore provided neither the thesis nor any substantial portion thereof may be printed or otherwise reproduced in any material form whatever without the author's prior written permission.



JAMES MAH

2403 - 112 A STREET
EDMONTON, ALBERTA
T6J - 4X4

DATE: JULY 31, 1992

"The art of medicine consists of amusing the patient while nature cures the disease."

Voltaire (1694-1778)

UNIVERSITY OF ALBERTA

FACULTY OF GRADUATE STUDIES AND RESEARCH

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled **Biochemistry and Histology of the Fetal Human Temporomandibular Joint Articular Disc** submitted by James Mah in partial fulfilment of the requirements for the degree of **Master of Science** in **Clinical Sciences and Oral Biology**.

Dr. Paul Major

Dr. Paul Scott

Dr. Ken Glover

Dr. Geoff Machin

DATE: JULY 28, 1992

To My Parents

For giving me the education that they could not have.

ABSTRACT

The purpose of this investigation was to study growth and development of the fetal human temporomandibular joint (TMJ) articular disc using biochemical and histologic methods. The discs of two fetuses aged 22 and 25 weeks intrauterine were studied. Light microscopy revealed that the disc is a highly cellular biconcave structure with a dense arrangement of collagen fibers. Cell density in the intermediate band was approximately 1.5 times that of either the anterior or the posterior bands. Capillaries containing red blood cells were observed along the inferior surface of the disc. Immunohistochemical staining for proteoglycans (PGs) and glycosaminoglycans (GAGs) revealed hyaluronic acid (HA), chondroitin sulphate proteoglycan (CSPG), and dermatan sulphate proteoglycan II (DSPG II) in the disc. Biochemical analysis of cell culture medium suggested that the major type of CS was C_6S. The CSPG core was estimated to be larger than 200 kDa. The relative percentages of total GAGs were calculated to be 32% HA, 42% CS, 26% DS. Very little KS was detected.

The fetal TMJ articular disc at 22 and 25 weeks of intrauterine development was found to have morphology and regional specialization similar to that of adult human and bovine discs, suggesting predominantly genetic determination of its growth, development and function.

ACKNOWLEDGEMENT*

To Dr. Ken Glover and Dr. Paul Major for helping me set my goals and for providing the assistance and opportunities to achieve them. They are examples that I will carry into my career.

To members of my thesis committee: Dr. Paul Major, Dr. Paul Scott, Dr. Ken Glover and Dr. Geoff Machin for their ideas and guidance.

To Dr. Takuo Nakano, Carole Dodd and Doug Marston for their great help and technical advice. I especially thank Dr. Nakano for his tireless patience and dedication. His committment extended beyond regular hours, often into evenings and weekends.

To the clinic instructors: Dr. Ron Mullen, Dr. Michael Pawliuk, Dr. Roland Haryett, and Dr. Subash Alimchandani for their invaluable instruction and their willingness to share their knowledge and experience.

To Dr. Paul Puszczak for his friendship, encouragement and valuable advice. I also thank Paul for providing much appreciated breaks from my studies and getting me out to many hockey games.

To my colleagues, for their tremendous support, assistance and their hockey pool money.

To my friends, who have remained close, in spite of the time dedicated to my studies, and who have always supported my efforts in life.

*** Funding for this project has been provided by the Reyburn E. McIntyre Fund, the Canadian Foundation for the Advancement of Orthodontic Education and the Faculty of Graduate Studies, University of Alberta.**

TABLE OF CONTENTS

Chapter I. LITERATURE REVIEW 1

1. Anatomy and Function of the Human Temporomandibular Joint Articular Disc 4

1.0. Introduction 4
1.1. Synovial Joints 4
1.2. Terminology of the TMJ Articular Tissue 6
1.3. Gross Anatomy of the TMJ Articular Disc 8
1.4. Micro Anatomy of the TMJ Articular Disc 12
1.5. TMJ Synovium and Synovial Fluid 13
1.6. The Articular Disc in Function 16
1.7. Shape of the Articular Disc 20
1.8. The Articular Disc in TMJ Dysfunction 22

2. Prenatal Development of the Human TMJ 36

2.1. Prenatal Synovial Joint Development 36
2.2. Prenatal Development of the Temporomandibular Joint 36
2.3. Composition of the Fetal TMJ Articular Disc 43

3. Glycosaminoglycans and Proteoglycans in Connective Tissue 46

3.1. Structure and Morphology of PGs and GAGs 46
3.2. Synthesis and Degradation of PGs and GAGs 56
3.3. Functional Aspects of PGs and GAGs 62
3.4. PGs and GAGs in Connective Tissue 64

Chapter II. STATEMENT OF THE PROBLEM 71

Chapter III. MATERIALS AND METHODS 76

1. Materials 81

1.0. Introduction 81
1.1. Fetal Human TMJ Articular Discs 82
1.2. Histology and Immunohistochemistry 84
1.3. Cell Culture 85
1.4. Isolation and Preparation of PGs and GAGs 86
1.5. Enzyme Linked Immunosorbent Assays 87
1.6. Uronic Acid Analysis 88
1.7. Cellulose Acetate Electrophoresis 88
1.8. Di Ferrante Method for Iduronic Acid 89
1.9. Thin Layer Chromatography 89
1.10. Polyacrylamide Gel Electrophoresis 89
1.11. Immunoblotting 90

2. Methods 90

2.1. Removal of the Articular Discs 90
2.2 Histology and Immunohistochemistry 92

2.3. Cell Culture 95
2.4. Isolation and Preparation of PGs and GAGs 99
2.5. Enzyme Linked Immunosorbent Assays 103
2.6. Uronic Acid Analysis 104
2.7. Cellulose Acetate Electrophoresis 105
2.8. Di Ferrante Method for Iduronic Acid 106
2.9. Thin Layer Chromatography 107
2.10. Polyacrylamide Gel Electrophoresis 107
2.11. Immunoblotting 108

Chapter IV. RESULTS 109

1. Histology and Immunohistochemistry 112
1.1. Histology 112
1.2. Immunohistochemistry 125
2. Enzyme Linked Immunosorbent Assays 134
2.1. Chondroitin Sulphate Proteoglycan 135
2.2. Dermatan Sulphate Proteoglycan II 144
2.3. Keratan Sulphate Proteoglycan 149
3. Uronic Acid Analysis 151
3.1. Uronic Acid Content 151
4. Iduron[illegible] Acid Analysis 152
4.1. Iduronic Acid Content 152
5. Cellulose Acetate Electrophoresis 154
5.1. GAG Representation 154
6. Thin Layer Chromatography 162
6.1. Chondroitin Sulphate Representation 162
7. Gel Electrophoresis and Immunoblotting 164
7.1. Polyacrylamide Gel Electrophoresis 164
7.2. Immunoblotting 166

Chapter V. DISCUSSION 167

1. Microscopy of the Fetal TMJ Articular Disc 170
1.1. Histology 170
1.2. Immunohistochemistry 174
2. Analysis of Cell Culture Medium 176
2.1. Enzyme Linked Immunosorbent Assays 176
2.2. Uronic Acid Analysis 180
2.3. Iduronic Acid Analysis 181
2.4. Cellulose Acetate Electrophoresis 182
2.5. Thin Layer Chromatography 183
2.6. Gel Electrophoresis and Immunoblotting 184
3. Glycosaminoglycans and Proteoglycans of the Fetal TMJ Articular Disc 185
3.1. Hyaluronic Acid 185

3.2. Chondroitin Sulphate 186
3.3. Dermatan Sulphate 188
3.4. Keratan Sulphate 188
4. Conclusion 190
5. Future Studies 192
5.1. Growth and Development Studies 192
5.2. Studies of the TMJ Articular Disc in Dysfunction 194
5.3. Applied Studies 195

Chapter VI. BIBLIOGRAPHY 196

LIST OF TABLES

Table #	Description	Page #
III.1.1.1.A.	Medical History of the Fetal Specimens	83
III.1.4.A.	Description of Cell Culture Medium	87
III.2.2.2.A.	Antibodies for Immunohistochemistry	93
III.2.5.A.	Antibodies used for ELISA	104
IV.1.1.4.A.	Spectral Absorbance of Alcian Blue Stained Sections	123
IV.1.2.1.A.	Chondroitin Sulphate Staining in Tissue Sections	126
IV.1.2.2.A.	Dermatan Sulphate Proteoglycan II Staining in Tissue Sections	128
IV.1.2.3.A.	Keratan Sulphate Staining in Tissue Sections	130
IV.1.2.4.A.	Hyaluronic Acid Staining in Tissue Sections	132
IV.2.0.A.	Description of Cell Culture Medium	134
IV.2.1.A.	CSPG /100 ml Culture Medium	135
IV.2.2.A.	DSPG II /100 ml Culture Medium	144
IV.3.1.A.	Uronic Acid /100 ml of Unconcentrated Culture Medium	151
IV.4.1.A.	Iduronic Acid /100 ml of Unconcentrated Culture Medium	153

LIST OF FIGURES

Figure #	Description	Page #
I.1.3.A.	Structure of the TMJ	8
I.1.3.B.	Sagittal View of the TMJ	10
I.1.6.A.	Shear Forces Generated During Mandibular Opening	17
I.1.6.B.	Separation of Forces with a Disc	17
I.1.6.C.	Upper and Lower Wedge-Shaped Spaces Trap Synovial Fluid	18
I.1.6.D.	Distortion of Fibrocartilage on Both Articulating Surfaces	19
I.1.6.E.	Insertion of an Articular Disc	20
I.1.8.2.A	Partial Anteromedial Disc Displacement	26
I.1.8.2.B.	Anteromedial Displacement with Reduction (Closed Position)	26
I.1.8.2.C.	Anteromedial Displacement with Reduction (Opening)	27
I.1.8.2.D.	Anteromedial Displacement with Reduction (Open Position)	27
I.1.8.2.E.	Anteromedial Displacement without Reduction (Closed Posit.)	29
I.1.8.2.F.	Anteromedial Displacement without Reduction (Opening)	30
I.1.8.2.G.	Anteromedial Displacement without Reduction (Open Position)	30
I.1.8.2.H.	Elongation and Herniation of Posterior Attachment	31
I.1.8.3.A.	Adhesive Disc Hypomobility (Closed Position)	33
I.1.8.3.B.	Adhesive Disc Hypomobility (Restricted Translation)	33
I.1.8.4.A.	Dislocation (Closed Position)	34
I.1.8.4.B.	Dislocation (Opening)	35
I.1.8.4.C.	Dislocation (Open Position)	35
I.2.2.A.	Formation of the Temporomandibular Joint	38
I.3.1.A.	Cartilage Proteoglycan Aggregate	48
I.3.1.B.	Model of Cartilage Proteoglycan	50
III.1.0.A.	Flowchart of Experimental Methods	80
IV.2.1.A.	ELISA for CS in DEAE1	137
IV.2.1.B.	ELISA for CS in DEAE2, 3, 4	138
IV.2.1.C.	ELISA for CS in DEAE5, 6	139
IV.2.1.D.	ELISA for C_4S, C_6S, DSPG II and HA	140
IV.2.1.E.	ELISA for C_4S, C_6S	141
IV.2.1.F.	ELISA for C_6S and CSPG (First Trial)	142
IV.2.1.G.	ELISA for C_6S and CSPG (Second Trial)	143
IV.2.2.A.	ELISA for DSPG II in DEAE1	145
IV.2.2.B.	ELISA for DSPG II in DEAE2, 3, 4	146
IV.2.2.C.	ELISA for DSPG II in DEAE5, 6	147
IV.2.2.D.	ELISA for DSPG II in FCS	148
IV.2.3.A.	ELISA for KSPG in DEAE5	150
IV.5.1.A.	DEAE1 (Phosphate Buffer)	154
IV.5.1.B.	DEAE1 with *S.* Hyaluronidase	155
IV.5.1.C.	FCS	155
IV.5.1.D.	DEAE1 (Pyridine-Acetate Buffer)	156
IV.5.1.E.	DEAE2	156

IV.5.1.F.	DEAE2 with *S.* Hyaluronidase	156
IV.5.1.G.	Standard with *S.* Hyaluronidase	157
IV.5.1.H.	DEAE3	157
IV.5.1.I.	DEAE4	157
IV.5.1.J.	DEAE4 with *S.* Hyaluronidase	158
IV.5.1.K.	DEAE5	158
IV.5.1.L.	DEAE5 with *S.* Hyaluronidase	158
IV.5.1.M.	DS with Chondroitinase B	159
IV.5.1.N.	DEAE5 with Chondroitinase B	159
IV.5.1.O.	DEAE6	159
IV.5.1.P.	DEAE6 with *S.* Hyaluronidase	160
IV.5.1.Q	DEAE6 with Chondroitinase B	160
IV.6.1.A.	DEAE1 - Thin Layer Chromatography	162

LIST OF PLATES

Plate #	Description	Page #
III.2.1.1.A.	TMJ Articular Disc from a Human Fetus	92
III.2.3.A.	Tissue Explant	96
III.2.3.B.	Confluent Monolayer of Cells	97
IV.1.1.1.A.	TMJ Articular Disc - Haematoxylin and Eosin (4X)	112
IV.1.1.1.B.	TMJ Articular Disc - Haematoxylin and Eosin (100X)	114
IV.1.1.1.C.	Immunohistochemical Staining for Factor VIII-Related Antigen	115
IV.1.1.2.A.	TMJ Articular Disc - Masson's Trichrome (4X)	116
IV.1.1.2.B.	TMJ Articular Disc - Masson's Trichrome (100X)	117
IV.1.1.3.A.	TMJ Articular Disc - Taenzer-Unna Orcein (4X)	118
IV.1.1.3.B.	TMJ Articular Disc - Taenzer-Unna Orcein (100X)	119
IV.1.1.4.A.	TMJ Articular Disc - Alcian Blue (4X)	120
IV.1.1.4.B.	TMJ Articular Disc - Safranin-*O* (4X)	120
IV.1.1.4.B.	TMJ Articular Disc - Safranin-*O* (100X)	121
IV.1.1.5.A.	Alcian Blue Staining of Articular Disc and Human Skin	124
IV.1.2.1.A.	Chondroitin Sulphate Staining in Tissue Sections	127
IV.1.2.2.A.	DSPG II Staining in Tissue Sections	129
IV.1.2.3.A.	Keratan Sulphate Staining in Tissue Sections	131
IV.1.2.4.A.	Hyaluronic Acid Staining in Tissue Sections	133
IV.7.1.A.	Gel Electrophoresis and Immunoblotting of DEAE5 and 6	165

LIST OF ABBREVIATIONS

Ab (α):	antibody
Ag:	antigen
BSA:	bovine serum albumin
CAEP:	cellulose acetate electrophoresis
CPC:	cetylpyridinium chloride
CS:	chondroitin sulphate
CSPG:	chondroitin sulphate proteoglycan
Dab:	3,3'-diaminobenzidine tetrahydrochloride
ΔDi-0S:	2-acetamido-2-deoxy-3-*O*-(β-D-gluco-4-enepyranosyluronic acid)-D-galactose
ΔDi-4S:	2-acetamido-2-deoxy-3-*O*-(β-D-gluco-4-enepyranosyluronic acid)-4-*O*-sulfo-D-galactose
ΔDi-6S:	2-acetamido-2-deoxy-3-*O*-(β-D-gluco-4-enepyranosyluronic acid)-6-*O*-sulfo-D-galactose
DMB:	dimethylene blue
DMEM:	Dulbeccos modified Eagles medium
DMSO:	dimethyl sulfoxide
DS:	dermatan sulphate
EDTA:	ethylenediamine tetraacetate
EIA:	enzyme immunoassay
ELISA:	enzyme linked immunosorbent assay
FCS:	fetal calf serum
GAG:	glycosaminoglycan
HEPES®:	4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
KS:	keratan sulphate
NEM:	*N*-ethylmaleimide
PBS:	phosphate-buffered saline
PBST:	phosphate-buffered saline containing Tween 20
PDS:	proteodermatan sulphate
PG:	proteoglycan
Roccal®:	alkyldimethylbenzyl ammonium chloride
RIA:	radioimmunoassay
SDS:	sodium dodecyl sulphate
TBS:	tris-buffered saline
TEMED®:	*N,N,N'N'*-tetramethylethylenediamine
TMJ:	temporomandibular joint
Tween®:	polyoxyethylene sorbitan monolaurate

CHAPTER ONE

LITERATURE REVIEW

LITERATURE REVIEW

OUTLINE

1. Anatomy and Function of the Human Temporomandibular Joint 4

1.0. Introduction 4

1.1. Synovial Joints 4

1.2. Terminology of the TMJ Intra-Articular Tissue 6

1.3. Gross Anatomy of the TMJ Articular Disc 8

1.4. Micro Anatomy of the TMJ Articular Disc 12

1.5. TMJ Synovium and Synovial Fluid 13

1.6. The Articular Disc in Function 16

1.7. Shape of the Articular Disc 20

1.8. The Articular Disc in TMJ Dysfunction 22

1.8.1. Deviation in Form 23

1.8.2. Disc Displacements 24

1.8.3. Adhesive Disc Hypomobility 32

1.8.4. Displacement of the Disc-Condyle Complex 33

2. Prenatal Development of the Human Temporomandibular Joint 36

2.1. Prenatal Synovial Joint Development 36

2.2. Prenatal Development of the Temporomandibular Joint 36

2.3. Composition of the Fetal TMJ Articular Disc 43

3. Glycosaminoglycans and Proteoglycans in Connective Tissue 46

3.1. Structure and Morphology of PGs and GAGs 46

3.1.1. Hyaluronic Acid 53

3.1.2. Chondroitin Sulphate Proteoglycan 54

3.1.3. Dermatan Sulphate Proteoglycan II 55

3.2. Synthesis and Degradation of PGs and GAGs 56

3.3. Functional Aspects of PGs and GAGs 62

3.4. PGs and GAGs in Connective Tissue 64

3.4.1. PGs of the TMJ Articular Disc 66

3.4.2. HA and the TMJ Synovial Fluid 68

1. Anatomy and Function of the Human Temporomandibular Joint

1.0. Introduction

The temporomandibular or craniomandibular articulation is the articulation between the mandible and the cranium which is comprised of bilateral diarthrodial joints referred to as temporomandibular or craniomandibular joints. Diarthrodial joints are freely mobile and the bony elements are encapsulated and connected to one another by a fibrous joint capsule. The capsule is lined with synovial membrane and the joint cavity is filled with synovial fluid. For these reasons, these joints are also synovial joints (Norkin and Levangie, 1983; Mankin and Radin, 1985).

The temporomandibular joints are unique because they share one bone with bilateral articulations. The action of one side influences that of the other and their normal functioning requires a highly developed coordination. The paired joints, ligaments, and muscles must work in synchrony to act as one functional unit. Many would agree that the *temporomandibular joint (TMJ)* is the most complex, least understood, and unique synovial joint of the body.

1.1. Synovial Joints

Typical synovial joints have four basic features; the bones forming the joints are separate; they are held together with a capsular ligament; the capsule is lined by synovial

membrane and the bone ends are covered by articular cartilage (Osborn, 1982). The TMJ is a variation of this basic synovial joint. Interposed between the articulating surfaces of the TMJ is dense avascular fibrous connective tissue which is commonly referred to as the articular disc. Other variants include the sternoclavicular and the acromioclavicular joints which have their bony ends covered with fibrocartilage.

The presence of fibrous connective tissue on the articulating surfaces of the TMJ has often been interpreted as an indication that the TMJ is not a load bearing joint. Known load-bearing joints are lined by hyaline cartilage (Barnett *et al.*, 1961; Wright *et al.*, 1973). However, research over the last 15 to 20 years has accumulated indicating that the TMJ is a load-bearing joint (Hylander, 1985). It has been suggested that the peculiar developmental history of the TMJ may explain the fibrous tissue covering the articular surfaces. The development of the TMJ is described in the next section.

Synovial joints have no planar surfaces, and even the curved surfaces are not congruent resulting in an imperfect fit between opposing articular surfaces. As a result, stresses are not concentrated in one spot for long. There is a joint position where there is maximal matching of opposing articular surfaces. This position is referred to as the *close-packed* position, and it is at this time that the joint manages stresses most effectively (Thomas and Bronstein, 1991). The close-packed position for the TMJ is reported to be when the mandibular condyle is half-way along the posterior slope of the articular eminence (Thomas and Bronstein, 1991).

The design of a synovial joint may confer a tremendous range of articulating movement between the bones involved (Wright *et al.*, 1973). An ideal joint provides a

wide range of possible actions as well as a certain degree of stability. The TMJ articulation allows for two types and four directions of movement (Thomas and Bronstein, 1991). During mandibular opening, an initial rotary movement is seen up to mid-opening, when a second translatory or gliding movement is seen up to full opening. Rotational movements occur primarily in the inferior compartment, while the translatory or gliding movements occur primarily in the superior compartment (Bell, 1986, 1990), however, pure rotational and translatory movements probably do not occur in normal TMJ function (Thomas and Bronstein, 1991). Coordinative and synchronous control allows for jaw opening, lateral excursions, protrusion, and retrusion of the mandible. Combinations of these basic movements produce numerous mandibular movements. During these movements, both TM joints sustain a load, however, the load varies as jaw position changes. Some studies suggest that the contralateral or non-working side sustains the greater load when masticatory forces are applied (Hatcher *et al.*, 1986; Greaves, 1978; Farrar, 1971).

1.2. Terminology of the Temporomandibular Joint Intra-Articular Tissue

A considerable amount of literature concerning the tissues which separate the temporomandibular joint into an upper and a lower compartment has been reported. The terminology of this tissue varies according to individual interpretation and method of examination. Lack of agreement on its description and terminology creates ambiguity and confusion.

The term *intra-articular tissue* includes all tissues that separate the two

compartments of the TMJ (Mahdry, 1971). This tissue consists of (1) an anterior band, (2) an intermediate zone, (3) a posterior band, (4) a bilaminar zone, (5) a medial attachment, and (6) a lateral attachment.

Some authors use the term *articular disc* to include all the intra-articular tissue, while others consider the articular disc to be a component of the intra-articular tissue. The dense fibrous anterior part of the intra-articular tissue is considered by some authors (Bellinger, 1948; Markowitz and Gerry, 1949; Dixon, 1962; Boyer *et al.*, 1964; Orban, 1957; Sicher, 1955) to be the entire extent of the articular disc. Other terms such as the *disc proper* and the *working area of the disc* (Findlay, 1965) are also present in the literature.

Sicher (1955) does not consider the bilaminar zone to be part of the disc. He considers the disc to be the pressure-bearing part characterized by the absence of nerves, blood vessels and synovial covering.

For our purposes, the term *articular disc* refers to the dense fibrous parts of the intra-articular tissue described by Rees (1954) as the anterior band, the intermediate zone, and the posterior band.

The TMJ articular disc is often incorrectly referred to as a meniscus. A meniscus is semi-lunar in shape and is open on one side in relation to its position in the joint. As observed in the knee joint, the menisci only partially cover the articulating joint surfaces and thus are really only three sided. The normal TMJ disc is complete on all four sides covering the entire joint surface.

1.3. Gross Anatomy of the Temporomandibular Joint Articular Disc

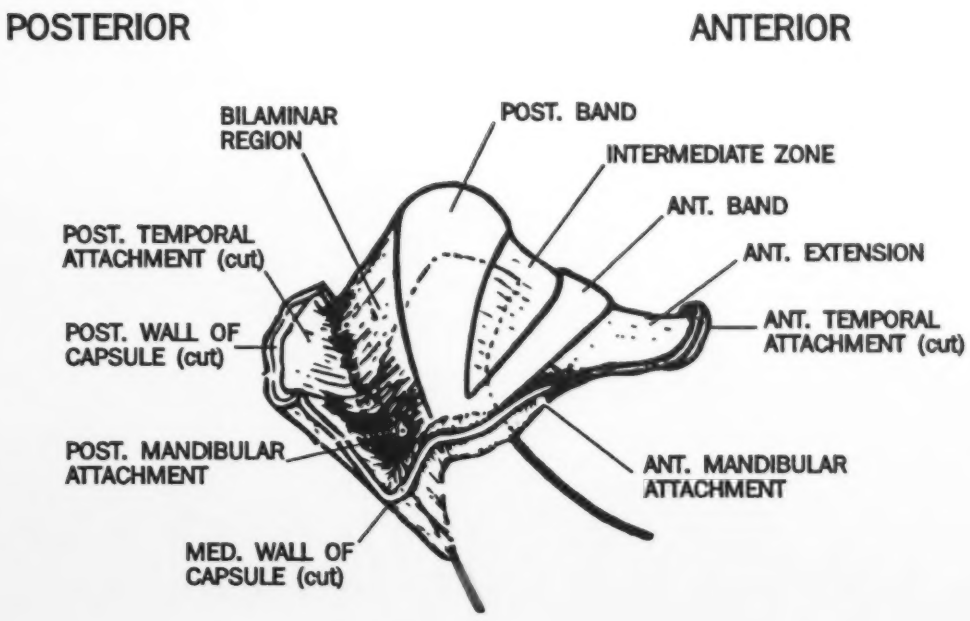


Figure I.1.3.A.
Structure of the TMJ
Classification and Terminology of the Structure of the TMJ - Lateral View of TMJ
(Modified from Rees, 1954)

Rees (1954) considered all the intra-articular tissue to be the disc. He described the articular disc as a dense sheet of fibrous tissue with a concave inferior surface and a superior surface which is convex posteriorly but saddle-shaped anteriorly, being slightly convex from side to side and slightly concave from anterior to posterior. He also reported that the disc is not equally thick throughout but exhibits four clearly defined transverse ellipsoidal zones (Figure 1.3.A.) which are termed; (1) the anterior band; (2)

the intermediate band; (3) the posterior band; and (4) the bilaminar zone.

Hansson *et al.* (1977) reported lateral thinning of the articular disc in the intermediate and posterior bands of the adult disc when compared to that of children, where the disc does not show this lateral decrease. They attributed this change to an increase in functional load on the disc.

Rees (1954) described the anterior band as moderately thick but relatively narrow from anterior to posterior. The intermediate band is observed to be much thinner than the others, and also narrower. The posterior band is seen as the thickest of the three and is also the widest from anterior to posterior. Anteriorly, the disc is attached to the articular eminence and to the lateral pterygoid muscle (Osborn, 1982). Posteriorly, the disc continues to become a thick double layer of vascularized connective tissue known as the bilaminar zone. The bilaminar zone consists of a superior stratum of fibroelastic tissue which is attached to the posterior wall of the glenoid fossa and the anterior lip of the squamo-tympanic fissure, and an inferior stratum of fibrous tissue which is attached to the borders of a triangular area behind and below the head of the condyle (Osborn, 1982). Posteriorly, the superior and inferior stratum are separated by a layer of loose connective tissues attached to the posterior wall of the joint capsule (Figure 1.3.B.). Both the bilaminar zone and the loose connective tissues contain a rich supply of nerves and blood vessels (Griffin, 1960; Rees, 1954). Zenker (1956) considered the posterior part of the intra-articular tissue to be part of the posterior wall of the joint capsule and described the entire structure as a *plastic retroarticular cushion*.

The medial and lateral aspects of the disc are not attached to the joint capsule but

are tightly bound by collateral ligaments to the medial and lateral poles of the condyle respectively (Krogh-Poulsen *et al.*, 1957). Consequently, the disc may rotate forward on the condyle, but it is prevented from anterior or posterior displacement as long as these ligaments are intact and functional. These ligaments are responsible for moving

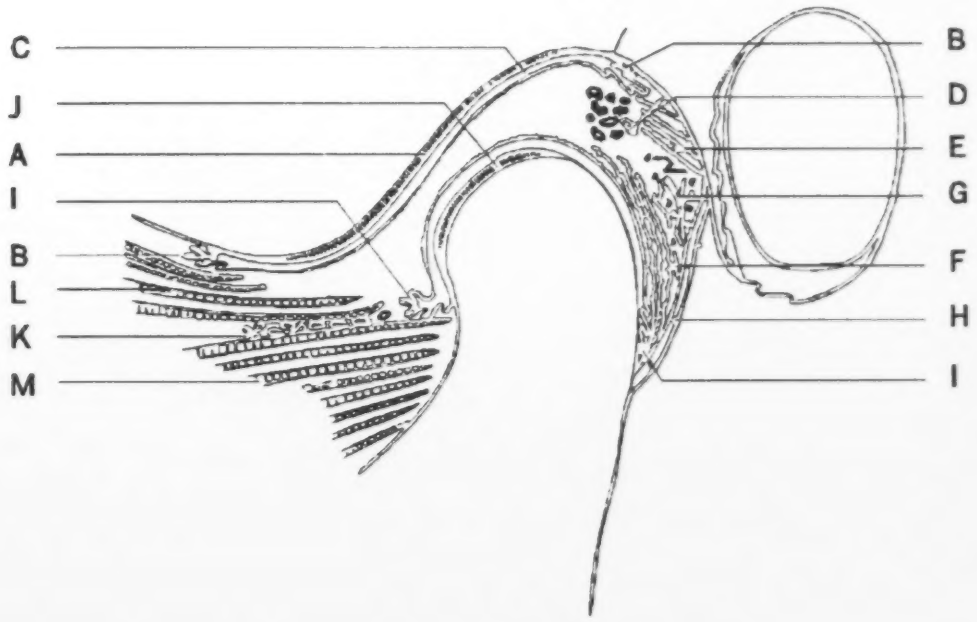


Figure I.1.3.B.
Sagittal View of the TMJ
(Adapted from Clark and Solberg, 1987)

A-Articular surface of the temporal bone, B-Synovial membrane of superior cavity, C-Superior cavity, D-Vascular Knee, E-Superior stratum of bilaminar zone, F-Inferior stratum of bilaminar zone, G-Loose areolar connective tissue, H-Posterior capsule, I-Inferior cavity synovial membrane, J-Articular surface of condyle, K-blood vessels, L-Superior belly of lateral pterygoid muscle, M-Inferior belly of lateral pterygoid muscle.

the disc along with the mandibular condyle during mandibular movements (Moffett, 1984). Krogh-Poulsen and Molhave (1957) and Mahdy (1971) emphasize that the intra-articular tissue is thicker medially than laterally.

The joint is encapsulated with a fibrous capsule, which is lined by a synovial membrane. This membrane is highly vascular and is continuous with the connective tissue of the capsule and of the disc. Each of the two compartments are lined with synovial membrane. The synovial membrane not only provides plasma filtrate for the synovial compartments but also produces synovial components necessary to create synovial fluid. The passive volume of the upper compartment measures approximately 1.2 ml, while the passive volume of the lower compartment measures approximately 0.9 ml of synovial fluid (Toller, 1974).

The shapes of the articular disc and synovium are altered during functional movement (Kaplan and Assael, 1991). On opening, the superior surface of the articular disc has a sigmoid shape in contrast to its shape in the closed position.

Anteriorly, a small portion of the superior head of the lateral pterygoid muscle is attached to the disc. The influence of the lateral pterygoid muscle on the articular disc is a subject of controversy. Contraction of this muscle has been suggested to protract the disc anteromedially or limit posterolateral retraction movements of the disc (Rees, 1954). Since the superior head of the lateral pterygoid muscle also attaches to the condylar head, it has also been suggested that this muscle has no special influence on movements of the articular disc relative to the condyle (Meyenberg, 1986; Wilkinson, 1988).

Öberg *et al.* (1966) described three component parts to the articular disc. These are: firstly, a thin central component characterized by its dense connective tissue, surrounded by the second component, a thick intermediate part (commonly known as the anterior and posterior bands of the disc) and thirdly, a peripheral component of loose connective tissue.

1.4. Micro Anatomy of the TMJ Articular Disc

The TMJ articular disc is composed of fibrocartilage (de Bont *et al.*, 1988). This cartilage differs from hyaline cartilage in that it has a greater proportion of dense collagen fibers. Acknowledging the high collagen content of the disc, it has also been described as dense fibrous connective tissue (Bhaskar, 1991). The disc is composed of type I collagen (Hirschmann and Shuttleworth, 1976). The matrix is sparse with numerous collagen fibers visible as large irregular bundles between groups of chondrocytes. The chondrocytes are generally arranged in rows parallel to the collagen bundles in pairs or singly (Jee, 1988). Fibrocytes (de Bont *et al.*, 1985) and a few of the chondrocytes (Rees, 1954) may be found randomly distributed throughout the disc.

The collagen fibers of the superficial layers of the anterior, middle, and posterior bands are oriented in both antero-posterior and mediolateral directions (Strauss *et al.*, 1960; Scapino, 1984). Scapino (1984) also described vertically oriented fibers in the posterior band[1]. The fibers in the central region of the anterior and posterior bands are

[1] Oriented at right angles to the disc surface

arranged in a random fashion. This arrangement imparts upon the disc the ability to resist forces in two directions (de Bont *et al.*, 1985). Shear and tensile forces in an antero-posterior direction are resisted by the superficial layers.

The fibrocartilage of the articular disc is primarily composed of chondrocytes, collagen fibrils, proteoglycans and water. The collagen fibrils and the proteoglycans form the extracellular matrix. Glycoprotein, small quantities of lipids and inorganic material may also be detected in the matrix. Although a few elongated fibroblasts may be found in the disc, the overall fibrous nature and limited vascularity of the disc suggest limited reparative capacity. In the adult, fibroblasts have been reported to differentiate into chondroid cells and eventually chondrocytes (Moffett, 1984). In rare instances, islands of hyaline cartilage may be observed in the disc. These cellular changes appear to be in response to mechanical loading (Bhaskar, 1976, 1991).

1.5. TMJ Synovium and Synovial Fluid

As in other synovial joints (Carson and Fox, 1985), the TMJ articular capsule is lined with a synovial membrane that folds to form synovial villi which project into the joint spaces (Bhaskar, 1991). In this manner, both the upper and lower joint compartments are lined with synovial tissue while the articulating surfaces of the temporal bone, the condyle, and the disc are not covered (Avery, 1987). Synovial tissue is most abundant on the superior and inferior retrodiscal lamina where it features multiple folds or villi that stretch upon translation of the condyle and disc (Goldman, 1991).

The joint capsule is lined with two layers of tissue. (Mankin and Radin, 1985).

The layer immediately adjacent to the joint surfaces is the synovial lining or intima. This layer ranges from a one cell layer to a four cell layer and in places, the cells are widely dispersed. The thinnest areas are those in closest contact with moveable joint components. The second layer is the subsynovial tissue, which contains cells like those of the first layer, but with a more developed connective tissue network and a richer blood supply. The joint capsule itself is relatively acellular, containing dense bands of collagen fibers.

Synovial tissue contains type A cells which resemble macrophages, type B cells which resemble fibroblasts and type C cells which are intermediate between the A and B types (Bhaskar, 1991). Approximately 80% of the synovial lining is composed of type B cells. Type B cells have a greater abundance of secretory granules and produce hyaluronic acid. Hyaluronic acid is thought to have a large role in providing friction-free joint movement (Harris, 1985; Carson and Fox, 1985). Type A cells function to remove joint debris while the purpose of the Type C cells has not been well established.

Harris (1985) argues that the assumption of Type A cells being macrophage-like and Type B cells being fibroblast-like may be overly simplistic as one cell may have more than one function, and in response to different stimuli cells can modulate their internal structure as their function changes. Type C cells or intermediate synovial cells have been described which have endoplasmic reticulum, Golgi complexes and vacuoles (Kelly *et al.*, 1983). Staining of ribosomal RNA is an acceptable index of synthetic activity of a cell. Normal synovium reveals staining in very few synovial cells. Following joint trauma, staining intensity is increased and is observed in greater numbers

of synovial cells (Waldenström *et al.*, 1978). Therefore, it may be reasonable to consider the synovial lining cell as one having multiple phenotypic possibilities (Harris, 1985).

Although the surface cells bind to the underlying matrix, they do not bind to any opposing tissue. Constant joint movement is thought to break down any cross links that may form between synovial lining cells and the opposing tissue (Drachman and Sokoloff, 1966). The synovial lining cells also secrete collagenase. It has been suggested that collagenase prevents both the formation of surface adhesions and the activation of the coagulation cascade by collagen fragments present on the tissue surface (Drachman and Sokoloff, 1966).

Friction-free joint movement[2] is achieved through two mechanisms (Harris, 1985). During joint movements under load, the hydrostatic pressure exceeds the intrinsic cartilage pressure, causing synovial fluid to be expressed. The fluid is forced ahead of the contacting joint surfaces. This process is known as *weeping lubrication*. During movements under little or no loading, *boundary lubrication* occurs via a glycoprotein named lubricin. This "lubricating protein" has been reported to bind to the surface of the cartilage while retaining water molecules at this site of interaction. In this manner, lubricin prevents the cartilage surfaces of a joint from making contact. The combination of these two mechanisms of joint lubrication has been termed *elasto-hydrodynamic*

[2] The coefficient of friction between the surfaces of synovial joints is approximately 1.002, where a friction-free coefficient would be 1.000 (Bewyer, 1989; Toller, 1961; Charnley, 1959).

lubrication (Weiss, 1988).

Synovial fluid is a dialysate of blood plasma which consists of a hyaluronic acid-protein complex with little or no sulphated glycosaminoglycans (Balazs *et al.*, 1967; Schmidt and MacNair, 1958). In conjunction with its role in joint lubrication, synovial fluid may act as a buffer against compressive loading forces simply through redistribution of forces (MacConail, 1950).

Normal synovial fluid behaves as a viscous fluid at low-strain frequencies, exhibiting thixotropic flow properties (Maulkin and Radin, 1989; Weiss, 1988). That is, the more slowly it flows, the more viscous it becomes. The viscosity of synovial fluid is largely determined by its hyaluronate content.

1.6. The Articular Disc in Function

Although Rees (1954) did not fully explain the significance of the varying thickness of the articular disc in its different zones, he argued that there was a correlation between structure and function of the articular disc. He offered two possible reasons for the varying thicknesses. Firstly, he suggested that the interposition of a thin zone between two thick zones should make the disc more flexible and enable it to alter its shape from concave below to convex below as it slides forward from the articular fossa on to the articular eminence. Secondly, he interpreted that the condyle, being separated in its retrusive position from the temporal bone by the thickest part of the disc, and by the thinnest part of the disc when it moves on to the articular eminence, is not constrained by the anatomical contours of the temporal surface but can move in a smooth

arc with its centre near the lingula.

Other authors have tried to rationalize possible explanations for the existence of an articular disc in a synovial joint. Hjortsjö (1953) observed that during mandibular opening and closing, the shear forces generated from rotation are compounded with those resulting from simultaneous translation (Figure 1.6.A.). He described the TMJ as an

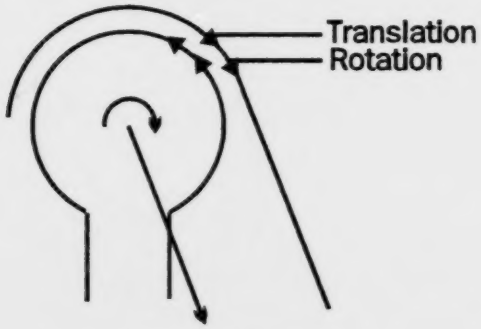


Figure I.1.6.A.
Shear Forces Generated by Translation and Rotation during Mandibular Opening
(Modified from Osborn, 1985)

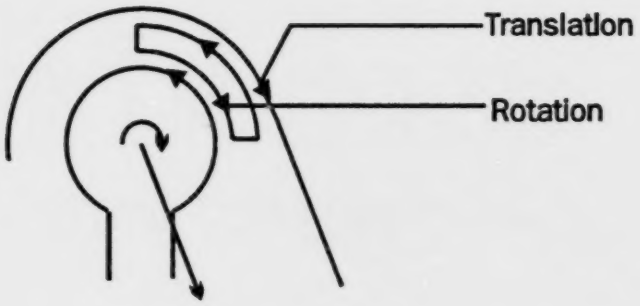


Figure I.1.6.B.
Separation of Translational and Rotational Forces with a Disc
(Modified from Osborn, 1985)

"accentuated grinding joint" and suggested that excessive tissue damage may be the result of such a design. Subsequently, he proposed that the role of the disc is to separate the two shear forces into different compartments in order to minimize potential damage (Figure 1.6.B.).

Toller (1961) compared the synovial fluid of the TMJ with that of other joints. He examined the lubricative properties of synovial fluid in relation to friction in synovial joints. He noted that synovial fluid is minimized on surfaces that are completely congruent but if a wedge-shaped space is present between the two surfaces, synovial fluid is trapped between them (Figure 1.6.C.). Therefore, he suggested that a role of the articular disc is to create superior and inferior wedge-shaped spaces in the TMJ in order to contain synovial fluid.

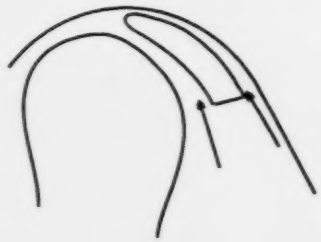


Figure I.1.6.C.
Upper and Lower Wedge-Shaped Spaces Trap Synovial Fluid
(Modified from Osborn, 1985)

The following advantages are outlined (Williams and Warwick, 1980; Jagger, 1980) for the presence of an interposing disc between articular surfaces; shock absorption; improvement of fit between the two non-congruent articular surfaces; facilitation of the combined movements; weight distribution over a larger surface area;

protection of the edges of the articular surfaces; and spread of synovial fluid for the purposes of lubrication. However, Osborn (1985) points out that shock absorption, improvement of fit under compression forces and distribution of weight could be accomplished by increasing the soft tissue thickness covering the bony surfaces rather than having an articular disc.

Osborn (1985) suggests that a possible functional advantage of having fibrous tissue in the TMJ is that it has the ability to distort considerably under compression, acting to distribute the load and minimize damage to the articular surfaces. However, if the design of the TMJ were such that the fibrous tissue simply lined the TMJ, there would be a potential flaw. Under load, the fibrous tissue covering the articular eminence would distort over the edges of the condyle (Figure 1.6.D.). This tissue would be damaged as the condyle "gouged" through it during mandibular grinding movement.

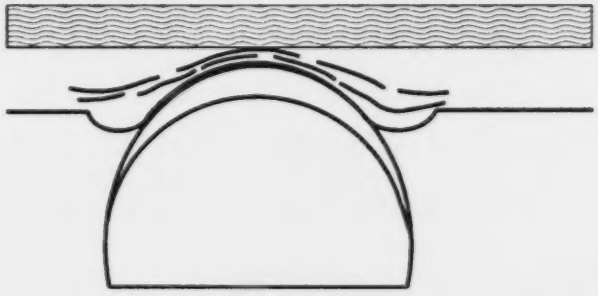


Figure I.1.6.D.
Distortion of Fibrocartilage on both Articulating Surfaces
(Adapted from Osborn, 1985)

An interposing articular disc would overcome this design flaw. During loading, the condyle deeply indents the disc, but the disc only marginally indents the articular

eminence (Figure 1.6.E.). This would permit a disc, even under load, to slide freely over the articular eminence. Therefore, Osborn (1985) proposes that the role of the articular disc is not to stabilize the condyle, but in fact, to "destabilize" it so that the loaded condyle and disc may glide freely over the articular eminence during mandibular grinding movements.

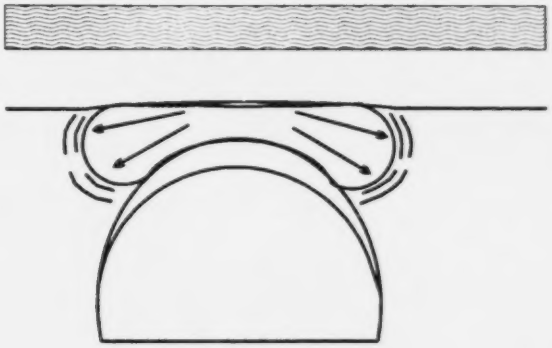


Figure I.1.6.E.
Insertion of an Articular Disc
(Adapted from Osborn, 1985)

1.7. Shape of the Articular Disc

The characteristic biconcave shape of the articular disc is of extreme importance in the equilibrium of the temporomandibular joint articulation (Bell, 1986). Bell (1990) depicts a scenario where the osseous components of the joint first come closer together during function. Joint forces are produced which are distributed and directed to cause a rotary movement of the disc bringing the thin central area between the areas under stress. This *self-centering* capability of the disc is thought to be vital to normal joint function (Moffet, 1984). Once the loading pressure is released, the joint space widens.

This permits the disc to rotate so its thicker portion occupies the superior joint space. When the disc loses its structural conformation and changes shape, it loses this self-centering capability (Moffet, 1984). The biconcave shape of the disc also contributes to resist disc displacement (Bell, 1986, 1990).

Osborn (1985) presents a biomechanical explanation for the shape of the articular disc. He argues that the disc is modelled in response to condylar forces which act to compress the disc into the articular eminence causing thinning in the centre and squeezing out a thickened anulus around its periphery. This shape could be accomplished and maintained by cellular remodelling of the extracellular matrix.

The thin central region of the articular disc as well as the fibrous layers covering the articulating surfaces of the condyle and temporal bone are devoid of blood vessels and nerves (Rees, 1954; Miles, 1962; Silva, 1969; Wright *et al.*, 1973). The absence of neurovasculature in these areas is consistent with the hypothesis that there is considerable reaction force along this portion of the joint (Hylander, 1991). Moffett (1984) reasons that joint loading may influence the shape of the disc. He offers case reports of articular discs with thin central regions due to "functional compression". However, in the same reports, no indication is given of what physiologic or pathologic process occurs to permit this "functional compression". In other words, without an identifiable biologic process, there is doubt whether "functional compression" occurs or if a thin central region of a disc may be a variation of normal.

1.8. The Articular Disc in TMJ Dysfunction

Disorders due to abnormal function of the articular disc are second only to the muscle disorders among TMD complaints (Bell 1986, 1990).

Dolwick (1983) termed "the abnormal relationship of the articular disc to the mandibular condyle, fossa, and articular eminence" as an internal derangement. Although this definition is commonly utilized, it is rather restrictive in that it is limited to disc displacement. Alteration in the morphology of the disc is also recognized as an important aspect of internal derangements (Heffez and Jordan, 1989). Therefore many authors have subsequently expanded upon this definition of internal derangement to include disc deformations. Kaplan and Assael (1991) propose the following classification for internal derangements of the TMJ:

I. Deviation in Form

- A. Frictional Disc Incoordination
- B. Articular Surface Defects
- C. Disc Thinning and Perforation

II. Disc Displacements

- A. Partial Anteromedial Disc Displacement
- B. Anteromedial Disc Displacement with Reduction
  1. Partial
  2. Complete
- C. Anteromedial Disc Displacement with Intermittent Locking
- D. Anteromedial Disc Displacement without Reduction

E. Anteromedial Disc Displacement with Perforation of Retrodiscal Tissue

III. Adhesive Disc Hypomobility

IV. Displacement of Disc-Condyle Complex

A. Subluxation

B. Dislocation

1.8.1. Deviation in Form

The structural morphology and integrity of the articular disc is essential to normal joint function. Optimum joint function dictates that the disc must be free to rotate on the condyle and translate against the eminence. The first group of internal derangements deals with deviation in form of the articular disc. **Frictional disc incoordination** is thought to be due to roughness of the superior surface of the disc or physical adhesions between the disc and eminence caused by excess pressure exhausting the lubrication of the articular surface of the eminence or disc (Mahan, 1985). Bell (1986, 1990) states that any movement of the disc-condyle complex that exhausts weeping lubrication is potentially damaging to the articular surfaces. According to Bell (1986, 1990), frictional disc incoordination may result from occlusal dysharmony that displaces the disc-condyle assembly during clenching. Bruxism, excessive biting force, and trauma with the teeth in occlusion are other causes. Frictional disc incoordination usually follows a prolonged period of joint immobilization. When the translatory joint movement begins, the disc may adhere to the eminence and resist normal movement. The resultant strain on the discal ligaments may predispose the patient to a true disc displacement.

An **articular surface defect** located on the articulating surface of the eminence or the superior surface of the disc, or both produces resistance to normal smooth translatory movement of the disc. Bell (1986, 1990) proposes that this type of defect may be the result of trauma to the mandible when the teeth are apart, "abusive use", and developmental and growth anomalies.

Static overloading of the TMJ may result in **thinning** of the articular disc and eventually, **perforation**. This type of perforation usually appears as a circular hole with fragmented borders in the intermediate zone of the disc. Disc fracture, leading to degenerative changes in the articular surfaces of the joint, has also been reported (Moffett, 1984b).

1.8.2. Disc Displacements

As discussed previously, the movement between the condyle and disc is a rotary movement which occurs during normal mandibular movements. Any other movements between the disc and the condyle are abnormal. The restrictive discal ligaments and the self-centering effect of the disc serve to preclude any sliding movements between it and the condyle. In order to allow for a sliding movement, not only must the borders of the disc be deformed, but also, the discal ligaments must be ruptured. In most instances, the posterior margin of the disc has been damaged, allowing for an anteromedial disc displacement (Bell, 1986, 1990; Okeson, 1985). From cadaver studies, Westesson (1984) reported that the articular disc is generally not displaced straight forward, but rotated about the medial attachment of the condyle. A further consequence of disc

displacement is elongation of the posterior attachment.

Rasmussen (1981) described progression of TMJ dysfunction in three phases. The first phase was characterized by clicking and lasted 3 to 6 years. The next phase involved painful locking and limited range of movement and lasted for approximately one year. The third phase featured crepitation and arthrosis. However, keep in mind that Rasmussen based this description on a longitudinal study and many generalizations were made to produce a model of progressive TMJ dysfunction and degeneration.

As many investigators have found (Bell, 1990; Lundh *et al.*, 1987; Pullinger and Seligman, 1987; Okeson, 1985; Farrar and McCarty, 1979), the clinical signs and symptoms of internal derangements are nonspecific for each stage and are not consistently reliable in determining the status of the disc-condyle relationship.

In a healthy joint, the center of the posterior band of the disc is observed to be in a 12 o'clock position with respect to the condyle when the teeth are in occlusion (Rees, 1954). The situation in which the end of the posterior band of the disc terminates anteriorly on the condyle in the closed joint position, is known as **partial anteromedial disc displacement** (Figure 1.8.2.A.). This type of displacement occurs as a result of thinning the posterior band of the disc in conjunction with slight elongation of the discal ligaments allowing for slight movement anteriorly on the condyle. The disc may also be slightly elongated as well as its posterior attachment (Ross, 1987).

This Figure Has Been Removed Due to the

Unavailability of Copyright Permission

Figure I.1.8.2.A.
Partial Anteromedial Disc Displacement
(From Kaplan and Assael, 1991)

Anteromedial Disc Displacement with Reduction features a further elongation of the discal ligaments and its posterior attachment tissues, allowing the disc to be malpositioned even further anteromedially, creating an obstruction to normal condylar translation (Figures 1.8.2.B.,C.). If reduction occurs late in the translatory cycle (Figure 1.8.2.D.), the prognosis for permanent stabilization of the disc on the condyle is extremely poor (Bell, 1986, 1990; Okeson, 1985). This condition is often referred to as a **reciprocal click**. A reciprocal click simply describes an opening and closing joint

This Figure Has Been Removed Due to the

Unavailability of Copyright Permission

Figure I.1.8.2.B.
Anteromedial Disc Displacement with Reduction (Closed Position)
(From Kaplan and Assael, 1991)

This Figure Has Been Removed Due to the

Unavailability of Copyright Permission

Figure I.1.8.2.C.
Anteromedial Disc Displacement with Reduction (Opening)
(From Kaplan and Assael, 1991)

This Figure Has Been Removed Due to the

Unavailability of Copyright Permission

Figure I.1.8.2.D.
Anteromedial Disc Displacement with Reduction (Open Position)
(From Kaplan and Assael, 1991)

sound and although it is often it is used as a diagnostic key to disc displacement with reduction, it may not be accurate (Westesson *et al.*, 1985).

It is likely that during this time, permanent deformation may already be occurring in parts of the disc that are anteriorly positioned. Westesson *et al.* (1985) reported a high incidence of disc deformation and irregularities of the articular surfaces associated with internal derangement. The most frequent type of deformation was thickening and

enlargement of the posterior band, but the major deformations involved the entire disc and were often associated with a perforation of the posterior attachment. They concluded that disc deformation was almost always preceded by disc displacement.

Using arthrography, Ronquillo (1988) reported a tendency for posterior condylar displacement in patients with disc displacement with reduction but not in symptomatic normals nor patients with disc displacement without reduction. However, great variability has been reported in the position of the condyle in relation to the glenoid fossa in both the normal population and TMJ patients (Cassaro and Pitini, 1988). As well, Herbosa *et al.* (1990) found no correlation between condylar position and TMJ symptoms.

Considerable variation in the position of the articular disc may exist as well. Arthrographic studies indicate that the position of the disc within the fossa is extremely variable whether the disc position is considered to be normal or abnormal (Brand *et al.*, 1989a).

Anteromedial disc displacement may be further subdivided into partial or complete displacements. Partial displacement involves only the lateral pole, allowing the disc to rotate about the medial pole. The medial part of the disc is sufficiently positioned to maintain the vertical dimension between the condyle and eminence. Complete displacement occurs when both the medial and lateral poles are involved, allowing the entire disc to be positioned anteriorly. Moreover, there is insufficient disc between articulating surfaces to maintain vertical joint space. The posterior band of the disc becomes relatively enlarged due to bulking of displaced tissue, increasing the likelihood

of progressing to disc displacement without reduction.

In **anteromedial disc displacement with intermittent locking**, the disc remains displaced for longer periods of time and the shape of the disc slowly changes from biconcave to biconvex, making passage of the disc over the condyle more difficult (Kaplan and Assael, 1991). At times the disc is in such a position that it does not readily return to its previous position. The patient must manipulate the mandible to work the disc back to its original position. At this stage, the retrodiscal tissue has thinned and deformed considerably. If reduction does not occur, then the condition progresses to **anteromedial disc displacement without reduction** (Dolwick, 1983; Farrar and McCarty, 1982), also commonly referred to as **closed lock**.

The normal relationship of the condyle, disc, and articular eminence is drastically changed (Figure 1.8.2.E.,F.). With the articular disc in this position, translation is reduced (Figure 1.8.2.G.). Anteromedial disc displacement without reduction is responsible for the majority of cases involving intracapsular restriction of mandibular

This Figure Has Been Removed Due to the

Unavailability of Copyright Permission

Figure I.1.8.2.E.
Anteromedial Disc Displacement Without Reduction (Closed Position)
(From Kaplan and Assael, 1991)

This Figure Has Been Removed Due to the

Unavailability of Copyright Permission

Figure I.1.8.2.F.
Anteromedial Disc Displacement Without Reduction (Opening)
(From Kaplan and Assael, 1991)

This Figure Has Been Removed Due to the

Unavailability of Copyright Permission

Figure I.1.8.2.G.
Anteromedial Disc Displacement Without Reduction (Open Position)
(From Kaplan and Assael, 1991)

movement (Kaplan and Assael, 1991; Bell, 1990; Okeson, 1985). In most instances, except cases involving overt trauma, there is a history of clicking followed by a period of limited opening (Farrar and McCarty, 1979b; Westesson, 1984; Schwartz and Kendrick, 1984).

For the purposes of treatment planning and prognosis determination, anteromedial disc displacement without reduction may be subdivided into an acute and a chronic substage (Kaplan and Assael, 1991). In general, if the disc has been displaced for six months or less, the condition may be referred to as acute. The prognosis of repositioning the disc decreases with longer periods of disc displacement (Howard, 1984). This condition may progress towards **anteromedial disc displacement with perforation of retrodiscal tissue**.

According to Farrar and McCarty (1979), an anteromedially displaced disc may cause the condyle to be displaced posteriorly and superiorly when the patient is in centric occlusion. Abnormal compressive loading on the anterior part of the posterior attachment may lead to structural changes in this area. Elongation of the retrodiscal lamina initially appears, and in some cases may lead to herniation of the posterior attachment (Figure 1.8.2.H.).

This Figure Has Been Removed Due to the

Unavailability of Copyright Permission

Figure I.1.8.2.H.
Elongation and Herniation of Posterior Attachment
(From Kaplan and Assael, 1991)

In other cases, however, compressive forces on the posterior attachment may lead to remodelling. The collagen fibers of the anterior part of the posterior attachment may become coarse (Blaustein and Scapino, 1983; Scapino, 1986) and compact masses of fibers may be found in relatively avascular areas of the posterior attachment.

At present, there is no explanation for the observation that some patients are able to adapt to these abnormal forces, while others continue on with the pathologic process.

1.8.3. Adhesive Disc Hypomobility

Arthrographic techniques have allowed workers to visualize adhesions in the upper and lower joint spaces. Adhesions may occur between the disc and condyle as well as between the disc and fossa (Bell, 1990; Okeson, 1989). Trauma with or without intracapsular bleeding has been implicated as a causative factor of joint adhesions (Kaplan and Assael, 1991; Okeson, 1989; Solberg and Clark, 1980). Thomas and Bronstein (1991) state that adhesions are the result of an inflammatory process. When they occur in the inferior joint space, the condyle and disc stick together, inhibiting normal rotational movement between them (Okeson, 1989). If the disc becomes fixed to the articular eminence, normal translation is prevented (Kaplan and Assael, 1991) (Figure 1.8.3.A.,B.).

This Figure Has Been Removed Due to the

Unavailability of Copyright Permission

Figure I.1.8.3.A.
Adhesive Disc Hypomobility (Closed Position)
(From Kaplan and Assael, 1991)

This Figure Has Been Removed Due to the

Unavailability of Copyright Permission

Figure I.1.8.3.B.
Adhesive Disc Hypomobility (Restricted Translation)
(From Kaplan and Assael, 1991)

1.8.4. Displacement of the Disc-Condyle Complex

During excessive opening, the condyle and the disc may be forced anteriorly beyond the normal limits of the translatory cycle. At this time, the disc cannot rotate any further posteriorly and the condyle continues to translate, causing a partial

dislocation or **subluxation** to occur between the disc and the articular eminence. The patient usually reports a history of jaw popping with wide opening during eating or yawning.

Reports in the literature (Beil, 1990; Okeson, 1985), indicate that an articular eminence with a short steep posterior slope may predispose a patient to subluxation and its sequel, complete dislocation. This anatomic feature is associated with brachyfacial types in which the occlusion is characterized by a deep bite.

According to Kaplan and Assael (1991), subluxation occurs when there is partial loss of contact between the disc and eminence, whereas **dislocation** involves collapse of the articular disc space (Figure 1.8.4.A.,B.,C.). Damage may occur to the discal attachment and the disc itself. Secondary elevator muscle spasm may perpetuate the dysfunction (Okeson, 1985, 1989).

This Figure Has Been Removed Due to the

Unavailability of Copyright Permission

Figure I.1.8.4.A.
Dislocation (Closed Position)
(From Kaplan and Assael, 1991)

This Figure Has Been Removed Due to the

Unavailability of Copyright Permission

Figure I.1.8.4.B.
Dislocation (Opening)
(From Kaplan and Assael, 1991)

This Figure Has Been Removed Due to the

Unavailability of Copyright Permission

Figure I.1.8.4.C.
Dislocation (Open Position)
(From Kaplan and Assael, 1991)

2. Prenatal Development of the Human Temporomandibular Joint

2.1. Prenatal Synovial Joint Development

Human joints, such as the knee, hip, elbow, and shoulder, all show the following general pattern of development (Moore, 1989). During the fifth week of intrauterine life, the skeletal elements are visible as a condensation of mesenchymal cells which differentiate into chondroblasts and form the cartilaginous precursor. By the seventh week, the future joint cavity is visible as a homogenous region of interzone cells. Ossification begins in the central portion of each bone and radiates towards the periphery. Cartilage remaining at the ends of the bones becomes the articular cartilage. During the eighth week, the cells of the interzone degenerate creating a definitive joint cavity. It is important to note that these joints form over a short period of time from a single blastema, and are complete by the end of the embryonic period.

2.2. Prenatal Development of the Temporomandibular Joint

The temporomandibular joint (TMJ) represents a unique structure in evolution, development, and function. It phylogenetically represents a new joint replacing the older one between the articulare (malleus) and the quadrate (incus) (Symons, 1952). The TMJ is truly a "special creation" in mammals and not an adaptation of a previously existing structure, a joint whose embryology repeats its phylogeny (Moffett, 1966).

The TMJ is one of the last joints in the body to appear and develop (Moffett,

1966; O'Rahilly and Gardner, 1978) and unlike the other synovial joints, the TMJ follows a markedly different order of development. It forms over a longer period of time from two separate blastemata, and is completed relatively late in fetal life (Baume, 1962, 1970). In contrast to the usual synovial joint, the TMJ is scarcely recognizable in the seventh week (Moffett, 1966). At this stage, the early mandibular body begins to form from bilateral intramembranous ossification centers in the integumental portions lateral to Meckel's cartilage.

Meckel's cartilage is the skeletal derivative of the first branchial arch that acts as a template for mandibular development. During embryonic life, when the rudimentary mandible does not yet contact the base of the skull, a temporary articulation for the lower jaw is formed with the posterior end of Meckel's cartilage (Moffett, 1966). Humphrey (1968) showed that active mouth opening in human fetuses could be observed as early as seven to eight weeks. This temporary joint is homologous with the jaw joint seen in non-mammalian forms.

Meckel's cartilage is present throughout the period of TMJ development until mid-fetal life at which time its dorsal end becomes incorporated into the middle ear as the malleus (Moffett, 1966) (Figure 2.2.A.). Membranous bone forms lateral to Meckel's cartilage in the region of the division of the inferior alveolar nerve into its mental and incisive branches beginning at the sixth week. Ossification spreads dorsally and ventrally to form the body and ascending ramus of the mandible up to the area of the lingula (Keith, 1982). Note that Meckel's cartilage does not become a component part of the mandibular condyle, and thus does not structurally contribute to formation

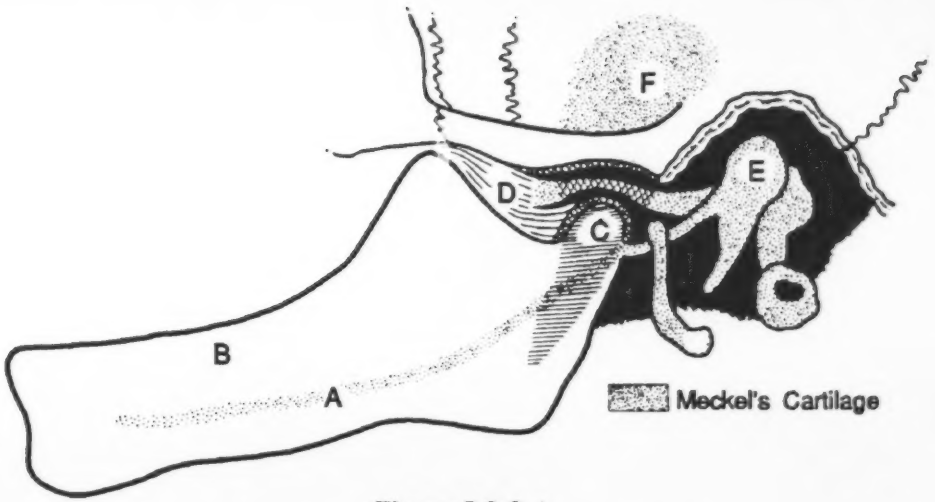


Figure I.2.2.A.
Formation of the Temporomandibular Joint
(Adapted from Mohl, 1988)
A-Meckel's Cartilage, B-Developing Mandible (Dentary Bone), C-Developing Condyle, D- Lateral Pterygoid and Fibrous Attachment extending to the Malleus, E-Malleus, F-Developing Temporal Component

of the TMJ.

During the eighth week, a condensation of mesenchymal cells, derived from neural crest cells, is observed on the dorsal end of the mandible (Keith, 1982). Over the next two weeks, these cells proliferate and differentiate to form the condylar cartilage. This cartilage is described as a carrot shaped structure, attached to the mandible and separate from the temporal component of the TMJ (Morimoto *et al.*, 1987) (Figure 2.2.A.).

During the tenth to twelfth weeks, the condylar cartilage undergoes significant

growth in size in a posterior and lateral direction to keep pace with the widening cranial base (Keith, 1982). Concomitant with this condylar cartilage growth is the formation of the joint capsule at eleven weeks, laterally and medially to the joint (Van der Linden *et al.*, 1987; Keith, 1982). Swallowing has been reported possibly as early as six weeks, but is commonly observed at 14.5 weeks (Sperber, 1976).

The TMJ develops from two blastemata initially separated from each other both spatially and chronologically (Baume, 1962, 1970). At the dorsal end of the developing mandible, the lower joint elements, or condylar components, develop from the condylar blastema. The condylar blastema, first recognizable at the eighth week, contributes exclusively to the development of the osseous condyle, the condylar cartilage, the tendon of the lateral pterygoid muscle including the anterior portion of the disc, and the capsular elements of the lower joint level. These components develop during the sixth to eighth weeks, before the appearance of the opposing glenoid structures.

The upper joint elements, or temporal components, are formed from the glenoid blastema in close relationship to the primordial quadrate-meckelian joint (Baume, 1970). The glenoid blastema is located at a more superior, posterior, and lateral level to the condylar blastema and is first recognizable at eight to nine weeks. The glenoid blastema contributes to the bony temporal joint structures, including the capsular elements of the upper joint level and the posterior portion of the disc. Note that the disc is a composite derivative of both condylar and glenoid blastemata.

There is some controversy as to the origin of the cells of the articular disc. Some researchers feel that these cells are derived from the lateral pterygoid muscle (Gaupp,

1911; Kjellberg, 1904; Vinogradoff, 1910; Symons, 1952; Moffett, 1957). While others such as Baume (1962, 1970), feel that they are derivatives of both condylar and glenoid blastema. And yet there are others (Haines,1947; Gardner and Gray, 1953; Barnett, Davies , and MacConaill, 1961) who feel they arise as a separate mesenchymal condensation. These investigators propose that the synovial membrane, subsynovial tissues and the discal ligaments develop from the vascular mesenchyme surrounding the primordial joint. Further yet, Harpman and Woollard (1938) feel that the lateral pterygoid tendon contributes only to the medial portion of the disc.

A disko-malleolar ligament was first described by Pinto (1962) in adults that extended from the medioposterosuperior border of the articular disc to the anterior process of the malleus. This finding has been confirmed by some investigators (Harpman and Woollard, 1938; Symons, 1952; Moffett, 1966; Coleman, 1970; Smeele, 1988) and denied by others (Baume, 1962, 1970; Aldolase 1966). Coleman (1970) was unable to confirm this finding in adults, but found supporting evidence in fetal specimens. In the fetal specimens this ligament was observed to extend from the posteromedial aspect of the disc and capsule, passing through the petrotympanic fissure, and attaching Meckel's cartilage to the malleus.

At the eighth to ninth week the condylar and glenoid blastemata are widely separated but gradually progress towards each other until they meet by the twelfth week. During this time, cavitation occurs by cell death and enzymatic breakdown, initially forming a lower joint compartment at ten weeks, soon followed by initial formation of an upper compartment by 11.5 weeks reaching completion by the fourteenth week. It

is during this time that positional relations are established between the mandible and the temporal bone, with easily observable superior and inferior joint cavities and the intervening articular disc (Moffett, 1966).

The cavities then increase in size to match the sizes of the articular fossa and mandibular condyle so that by 17.5 weeks, both cavities are present and fully match the sizes and shapes of the fossa and condyle. The shapes of the upper and lower joint cavities are reciprocal, the lower joint cavity is convex to match the shape of the condyle and the upper joint cavity is generally concave (Osborn, 1985).

Sperber (1989) states that this process of cavitation occurs by degradation rather than by enzymatic liquefaction or cell death. He further states that synovial membrane invasion may be a requisite for cavitation.

The role of active skeletal musculature for joint cavity formation and maintenance has also been studied. Scott (1951) investigated prenatal development of the sheep jaw joint and observed that cavitation coincides with early fetal jaw movements. In paralyzed joints an absence of cavity formation, fusion of the articulations, and skeletal deformity are observed, even in the presence of all the cellular components that form the TMJ (Drachman and Sokoloff, 1966; Murray and Drachman, 1969). Sperber (1989) states that the role of movement is to rupture the connective tissues separating the various minute spaces, allowing for their coalescence into functional cavities.

The biconcave shape of the articular disc has been reported to develop from compressive forces on the central portion of the disc as the condyle and articular

eminence assume their respective forms. Osborn (1985) states that although the articular disc does not originally exhibit a uniform thickness, its shape may be primarily due to the compressive forces to which it is subjected to during function. However, Van der Linden *et al.* (1987) found that the articular disc appears biconcave at 7.5 weeks. Furstman (1963) observed the mature morphologic shape of the disc at fourteen weeks as a thin central area increasing in thickness towards the peripheries. Strauss (1960) described the disc in the neonate as having the same basic biconcave shape as the adult. Wong *et al.* (1985) found that the shape of the articular disc at the time of initial delineation (13 to 17.5 weeks) resembled the shape of the adult articular disc. The weight of the scientific studies seems to suggest that the shape of the disc has a predominantly genetic determination although there is a role for functional pressures to contribute to the contouring and maintenance of the articulating surfaces.

Furstman (1963) states that once the main components of the TMJ are formed at fourteen weeks, no further histologic changes are observed except in size of these parts. Up to this point condylar growth has been through interstitial and appositional growth of cartilage. Endochondral bone formation begins at the fourteenth week and from this point on, the condyle increases in size exclusively by this method. Concomitant with growth of the condyle, intramembranous bone formation occurs in the temporal region to further develop the glenoid fossa.

Morimoto *et al.* (1987) felt that week 21 was the greatest turning point in TMJ development as this time is marked by the disappearance of Meckel's cartilage and all the components of the TMJ are easily identifiable at this stage.

There are currently very few studies pertaining to the development of the TMJ during the latter part of fetal life, but it is clear that there is an enormous amount of condylar growth in a superior, lateral, and posterior direction to keep up with the tremendous growth of the neurocranium.

2.3. Composition of the Fetal TMJ Articular Disc

As the inferior and superior compartments of the TMJ form from clefts in the coalescing condylar and temporal blastemata, the articular disc becomes noticeable as the interposed undifferentiated mesenchymal cells are converted to fibrous connective tissue (Symons, 1952; Furstman, 1963). Although the first appearance of the articular disc primordia has been described as early as 7 to 7.5 weeks as a deeper staining horizontal zone of mesenchyme running transversely within the block of tissue separating the articular fossa and the condylar elements, the earliest signs of collagen fibres in the disc are not seen until 10 to 10.5 weeks (Van der Linden *et al.*, 1987). These collagen fibres become more pronounced by approximately the twelfth week, and from the 19th to 20th week, the disc progressively becomes more fibrous in composition. From the fifteenth week through to full term, the disc increases in thickness and density by about five to six times (Morimoto *et al.*, 1987). The newly formed disc is described as having a thinner, avascular central region compared to the periphery (Wong *et al.*, 1985).

The development of the fibrocartilaginous articular disc reflects its intermediate nature between dense connective tissue and hyaline cartilage. During development,

fibrocartilage first appears as connective tissue (Symons, 1952; Furstman, 1963). The fibroblasts present in this tissue gradually transform into chondroblasts and secrete a thin layer of matrix around themselves. However, the secretion is limited and the collagen bundles never become incorporated into the sparse matrix (Jee, 1988). A perichondrium also fails to develop (Jee, 1988).

In sections of the adult human TMJ articular disc, the fibrous tissue has been described as consisting of an outer layer in which the fibre bundles run parallel with the articular surface and an inner layer in which the fibre bundles run obliquely (Keith, 1982; Strauss, 1960). Strauss *et al.* (1960), described two types of collagen fibres in the adult TMJ articular disc. Fine fibres having no particular orientation, run in all different directions forming a felt-like structure. On the other hand, the strong fibres are arranged in a delicate interlacing arrangement, seemingly oriented in a manner to resist tractional forces on the disc. A number of these fibres continue on into both the medial and lateral collateral ligaments. In the fetus, the disc shows the same basic structure as found in the adult (Furstman, 1963). This may suggest that the structure of the disc is to a large extent genetically determined.

Elastic fibres have been observed in the anterior and posterior bands from week 21 to full term (Morimoto *et al.*, 1987). Fine elastic fibres have been described in the peripheral portions of the articular disc in mice in the late fetal stages of development, when the major elements of the TMJ are present (Keith, 1982). By birth, abundant elastic fibres are to be found in the human TMJ articular disc. Strauss (1960) found elastic fibres intermingled amongst the collagen fibres. These fibres varied in amount

from area to area. They were quite numerous in the border areas, while fewer were found in the central portion of the disc. Very thick elastic fibres were found towards the posterior capsule. Keith (1979) studied fetal bovine articular discs and found the distribution of elastic fibres to be parallel to the collagen fibres and uniform throughout the different regions of the disc. He also found a band of thick coarse elastic fibres in the posterior aspect of the disc.

3. Proteoglycans and Glycosaminoglycans in Connective Tissue

Connective tissues form the architectural framework of the vertebrate body. The amount and distribution of connective tissue varies throughout the body. Large amounts may be found in organs such as skin and bone while only minor amounts may be found in the brain and spinal cord (Alberts, 1989). Connective tissues may be thought of as systems of insoluble fibrils and soluble polymers which work cooperatively to cope with the stresses of movement and to maintain shape (Scott, 1988). The fibrils resist tensile forces, while the interfibrillar soluble polymers resist compressive forces.

In connective tissues, the extracellular matrix is usually more plentiful than the cells and surrounds the cells on all sides, determining the tissue's physical properties. The composition of extracellular matrix is primarily water with a variety of polysaccharides and proteins (Hukins, 1984; Alberts, 1989).

3.1. Structure and Morphology of Proteoglycans and Glycosaminoglycans

The principal polysaccharides of the extracellular matrix are termed proteoglycans (PGs). They consist of a protein core with multiple covalently linked glycosaminoglycan (GAG) side-chains and an *N*- and/or *O*-linked oligosaccharide. The core protein of PGs are the least studied component of these molecules. The core protein sequence of most PGs remain unknown. Proteoglycans are large ubiquitous molecules that are composed

of a mixture of proteoglycan subunits and are usually found in the form of large macromolecular aggregates (Tsiganos *et al.*, 1971).

In general, the molecular weight of a cartilage PG core protein is 250,000 and measures approximately 300 nm in length (Hascall and Hascall, 1981). Compared to other large matrix proteins, this is considered to be a large molecule, such as a collagen α chain (MW $\approx$ 100,000) or fibronectin (MW $\approx$ 220,000). Considering that the core protein represents only 5-10% of the total mass of a PG molecule (Hascall and Riolo, 1972), the entire PG molecule is immense. Most of the remainder of the PG molecule consists of GAG chains. A typical cartilage proteoglycan may consist of as much as 95% carbohydrate by weight, most of which is due to the one to several hundred GAG chains, each averaging about 80 dissacharides long and usually without sialic acid. It is the GAG side-chains that are responsible for the bottle-brush appearance of cartilage PGs under the electron microscope.

Cartilage PGs are known to form very large aggregates in the extracellular matrix. There may be 30 or more PGs that are linked to a HA chain, which itself is a GAG (Hardingham and Muir, 1972a) (Figure 3.1.A.). The chain length of the HA molecule determines the size of the PG aggregate as it may bind up to 100 PG molecules, creating extremely large aggregates (MW of 2×10^8) (Hardingham, 1986). Binding of PGs to the HA complex may be further stabilized by a link protein (Hardingham and Muir, 1972) which couples the PG s to the HA molecule (Muir, 1981). Under physiological conditions, this appears to be an irreversible process (Hardingham, 1979).

Another group of PGs are the small interstitial PGs, which frequently contain DS (Hascall, 1986). Unlike the cartilage PGs, these molecules contain only small chains and are found in connective tissues with fibrillar collagen matrices. In this group are included proteochondroitin sulphate and dermatan sulphate proteoglycan II (proteodermatan sulphate). These members of the class of small PGs are widely distributed in connective tissues (Scott *et al.*, 1989; Poole, 1986) and are associated with the control of orientation and growth of collagen fibers (Scott and Nakano, 1989; Kresse *et al.*, 1986). In addition, these small PGs do not aggregate with HA (Heinegärd *et al.*, 1981a). Compared to the cartilage PGs, these PGs have not been as well studied.

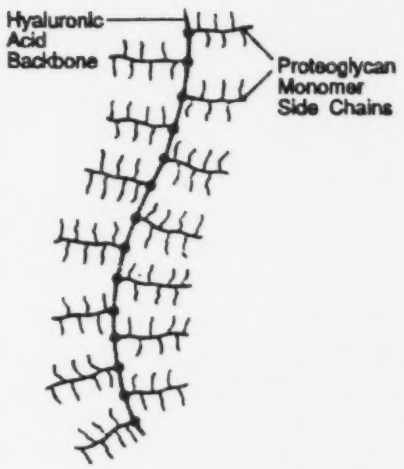


Figure I.3.1.A.
Cartilage Proteoglycan Aggregate

PGs that form aggregates have been referred to as high buoyant density PGs and those PGs that do not aggregate have been termed low buoyant density PGs (Thonar and Kuettner, 1987). In the group of high buoyant density PGs, there are two subgroups of PGs; one exhibiting a high C_6S content and the other one with a relatively increased KS content (Frazen, 1984; Heinegärd *et al.*, 1981a). Although most of the high weight PGs are aggregates, there are complexes that do not form aggregates (Frazen, 1984; Stockwell, 1979; Brandt and Muir, 1971; Tsiganos and Muir, 1969). These non-aggregating types are characterized by increased protein content (Heinegärd and Hascall, 1979).

At one time, it was thought that the function of PG aggregation was to immobilize them in the extracellular matrix, however, at present, other functions have been attributed to PG aggregation. Destruction of aggregates may alter the physical properties of cartilage allowing PGs to diffuse into the tissue, leaving endogenous collagenase to remain active in the matrix (Muir, 1981). Once the components of the aggregate are separate, they are no longer resistant to the destructive activity of collagenase (Harper and Muir, 1981).

Proteoglycans are distributed in all tissues and function not only as supporting structures, but also as mediators of events that characterize development and various diseases (Wight and Mechan, 1987). Although PG aggregation appears to be an important feature of cartilage, the relationship between this mechanism and cartilage function is not known. It has been suggested that aggregation of PGs immobilizes them within the network of collagen fibers creating a large osmotic swelling pressure to impart physical properties of stiffness and resiliency to cartilaginous tissues (Hardingham *et al.*, 1986). These properties enhance the ability of cartilage to resist compressive deformation (Hardingham, 1979).

Proteoglycans are well known for their tremendous variability. Firstly, many different types of proteoglycans may be found in the same tissue. Secondly, each type of proteoglycan is polydisperse; molecules of different size and charge exist. In addition, the number of disaccharide units within a particular type of GAG varies, as well as its sulphate content. Moreover, the spatial arrangement of hydroxyl, sulphate, and carboxyl side groups along the side chains differ (Alberts *et al.*, 1989). As a result of this

variation, the charge density and molecular weight of a particular type of proteoglycan can only be expressed as an average for the whole sample and the figure presented may differ according to technique used for preparation and measurement. At present, PGs are classified according to their GAG side chains, however, in the future, recombinant DNA techniques may allow identification of the core protein sequence and lead to development of a classification scheme based on core proteins as well as the constituent GAGs.

Proteoglycans may be comprised of one type of GAG or may contain several types of GAGs on the same protein core (Bartold, 1987; Silbert, 1982; Stockwell, 1979; Tsiganos and Muir, 1969). Variation also exists in the proportion of carbohydrate to protein over all the chains per core molecule (Bartold, 1987). Studies of cartilage PG's have shown that a single PG subunit consists of one core protein to which approximately 250 carbohydrate chains are attached (Hunziker and Schenk, 1987). Studies utilizing enzymatic and chemical degradation methods have lead to the development of a model of a cartilage PG subunit (Heinegärd and Axelsson, 1977). The proposed model is a molecule in which there are three

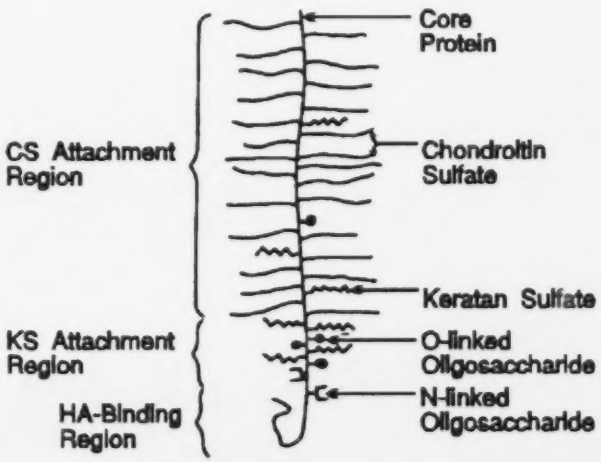


Figure I.3.1.B.
Model of Cartilage Proteoglycan

regions: a relatively constant region rich in KS chains; a more extensive variable CS-rich region containing other types of GAGs; and a HA binding region (HABR) containing a few short KS chains, which is also constant (Figure 3.1.B.).

Glycosaminoglycans are long, unbranched polysaccharide chains consisting of repeating disaccharide units. One of the two repeating sugar residues is always a hexosamine (*N*-acetylglucosamine or *N*-acetylgalactosamine). The hexosamine is usually sulphated. The alternating sugar is uronic acid (glucuronic acid or iduronic acid), except in KS where it is galactose. Unlike the hexosamine, which is either glucosamine or galactosamine, an individual chain may have a mixture of uronic acids (Alberts *et al*, 1989; Silbert, 1982; Hay, 1981).

GAGs are highly negatively charged due to the sulphate or carboxyl groups on most of the disaccharide units (Stockwell, 1979). Glycosaminoglycans are categorized into four main groups according to their sugar residues, type of linkage between these residues, and the number and location of sulphate groups (Alberts *et al.* ,1989). The groups are: hyaluronic acid (HA), chondroitin sulphate (CS), dermatan sulphate (DS), heparin sulphate (HS) and heparin, and keratan sulphate (KS).

With the exception of HA, GAG chains are not lone- standing in tissues, that is, they are always found bound to core proteins as components of proteoglycans. GAGs are secured to the protein core of PGs via a neutral trisaccharide (Gal-Gal- Xyl) located at the reducing end of the GAG chain (Hardingham, 1981).

The molecular weights of GAG chains varies within a range of 1-2 x 10^4, except

HA which is considered to be the largest at a molecular weight of 10^4 to 10^7. In spite of this, GAGs account for only approximately 10% of the weight of the total amount of fibrous proteins in connective tissue (Alberts *et al.*, 1989).

GAG chains are highly negatively charged and as a result, do not fold up into the compact globular domains that polypeptide chains typically form. As well, they are strongly hydrophilic. This results in GAG chains that are highly extended (often referred to as random-coil confirmations) and occupy an enormous volume relative to their size. The negativity of the GAG chains attracts cations such as Na^+ that increase the osmotic pressure in the immediate area, resulting in large amounts of water being held in the matrix. Gel formation occurs at very low concentrations. A swelling pressure or turgor is created that allows the matrix to withstand compressive forces (Evered and Whelan, 1986; Hascall and Hascall, 1981). The amount of swelling is limited by the tension exerted by the collagen fibrils. Because of this mechanism, collagen fibers are under continuous stress even when a joint is at rest (de Bont, 1985).

Gel formation causes the GAG chains to occupy most of the extracellular space. Proteoglycan gels exhibit varying pore sizes and charge density. These features allow the PG gel to function as a sieve to regulate the flow of molecules and cells according to their size and/or charge (Granström and Linde, 1973; Maroudas, 1974). This PG function has been observed in the basal lamina of the kidney glomerulus, which filters molecules passing from the bloodstream into the urine. In short, PG gels provide mechanical support of tissues, while allowing diffusion of water-soluble molecules and the migration of cells.

In view of the complexity, extensive variation, and location of most GAG complexes, it is most probable that they have more than one singular function (Gallagher *et al.*, 1986). Moreover, it is likely that few functions of GAGs are known or even suspected (Poole, 1986). GAG chains have been reported to play a role in the regulation of the extracellular matrix production and organization as well stabilization of the differentiated state of cells (Bronsson *et al.*, 1988; Toole, 1981).

3.1.1. Hyaluronic Acid

Hyaluronic acid (HA) is also commonly referred to as hyaluronate or hyaluronan. HA is considered to be a relatively simple molecule usually consisting of several thousand repeating nonsulphated disaccharide units. HA has been associated with water content of tissues with the level of HA indicating the degree of hydration of the particular tissue (Toole, 1982). Various amounts of HA are found in all mammalian tissues and fluids, but are especially abundant in early embryos. HA is considered to be the most widespread GAG.

Hyaluronic acid is associated with several biological processes, including a vital role in aggregation of cartilage PGs (Hascall, 1986). HA is also thought to have a key role in tissue morphogenesis and repair (Laurent and Fraser, 1986; Toole, 1981). The production and enzymatic degradation of HA is postulated to control cell migration and cell differentiation in developing and remodelling tissues (Toole, 1981). The production of HA may be correlated with the formation of a swelling network-like gel to facilitate cell migration during periods of development and cellular remodelling. Following the

cessation of cellular migration, there appears to be increased hyaluronidase[3] activity (Toole, 1981) possibly acting to dismantle the network-like gel.

3.1.2. Chondroitin Sulphate Proteoglycan

Chondroitin sulphate proteoglycan (CSPG) is found in large amounts in hyaline cartilage as well as many other tissues (Rosenberg and Schubert, 1970). As well, chondroitin sulphate (CS) is found widespread in connective tissues where they may exist in many hybrid forms where different forms exist in the same or different molecule (Bartold, 1987; Seno *et al.*, 1975; Hoffman *et al.*, 1956).

The repeating disaccharide unit of the chondroitin sulphates consists of glucuronic acid and *N*-acetylgalactosamine with the glucuronidic linkages being (β1→3) and the hexaminidic linkages being (β1→4). Chondroitin sulphate is categorized as two forms; chondroitin 4 sulphate (C_4S) and chondroitin 6 sulphate (C_6S) depending on which hydroxyl group in the *N*-acetylgalactosamine unit is esterified with the sulphate (Carney and Muir, 1988; Rodén, 1980). Historically, these two major types of chondroitin sulphate were once referred to as chondroitin sulphates A and C (Meyer, 1956). It is now known that the difference between type A and C is the position of the sulphate group, with type A being sulphated at position 4 of the galactosamine residue, and type C being sulphated at position 6. Subsequently, the more appropriate names of chondroitin 4 sulphate and chondroitin 6 sulphate have been used.

---

[3] Hyaluronidase is an enzyme that degrades HA.

CSPG's are found in regions of tissues that are subjected to large amounts of stress, such as cartilage and tendon and is thought to play a role in dissipating load applied to the tissue (Poole, 1986; Vogel and Heinegärd, 1985). It has also been suggested that the large amounts of CS in cartilage may indicate that this GAG chain is important in cartilage formation (Toole, 1981).

3.1.3. Dermatan Sulphate Proteoglycan II

Dermatan sulphate differs from CS by the presence of a *L*-iduronic acid rather than *D*-glucuronic acid, however, both types of uronic acids have been reported in the same molecule (Habuchi *et al.*, 1973; Bartold, 1987).

Although dermatan sulphate proteoglycan-II (DSPG II/PDS/decorin) is reported to be the predominant PG of skin (Damle *et al.*, 1982; Pearson and Gibson, 1982) and tendon (Vogel and Heinegärd, 1985), it has been described in virtually all fibrous connective tissues. DSPG II consists of a protein core of 330 (Day *et al.*, 1987) or 329 (Krusius and Ruohslahti, 1986) amino acids to which is attached a single DS chain at residue #4 (serine) (Chopra *et al.*, 1985) and 3 *N*-linked oligosaccharides (Scott and Dodd, 1990; Scott *et al.*, 1989).

For the most part, DSPG II is distributed in the extracellular matrices of connective tissue as a small interstitial PG (Heinegärd *et al.*, 1985). An important biological property of DSPG II is that it binds non-covalently to collagen fibrils and fibronectin and inhibits fibrillogenesis *in vitro*, as well as inhibiting the adhesion of

fibroblasts to fibronectin. DSPG II has also been observed to inhibit collagen fibril growth *in vitro* (Vogel and Trotter, 1987; Vogel *et al.*, 1984) and is associated with specific sites along the collagen fibrils (Scott, 1986). In addition, DSPG II has been shown to be associated with the surfaces of collagen fibers in the bovine TMJ articular disc (Nakano and Scott, 1989; Scott 1988) and may have a role in inhibiting mineral deposition within the collagen fibrils on the normally uncalcified tissues (Scott,1988). Furthermore, through the enhancement of gap-junctions, DSPG II augments cell-cell communication (Rosenberg *et al.*, 1986).

Poole *et al.* (1986) found DSPG II in adult bovine metacarpal-phalangeal joints to be present mainly in the superficial zones of the cartilage, while the molecule was found to be uniformly distributed in bovine fetal articular and epiphyseal cartilages. However, they also found that shortly after birth, the distribution of DSPG II was limited to the superficial and middle zones of articular cartilage. Lastly, they detected DSPG II on collagen fibrils of tissues such as skin, tendon, ligament and arterial vessels and suggested that DSPG II may play an important role in determining the sizes and tensile properties of collagen fibrils.

3.2. Synthesis and Degradation of PGs and GAGs

In view of the complexity of the structure of a proteoglycan molecule, it is apparent that assembly of the PG requires the coordination of a number of specialized biosynthetic pathways involved in post-translational processing. There are probably several mechanisms working in different regions of the cell to ensure that the different

constituents are placed on the PG core in the proper sequence and that the partially completed molecule proceeds through the intracellular sites in an orderly fashion.

All GAGs are synthesized on a protein core primer, with the exception of HA (Sooner and Thompson-Pletscher, 1986). The site of HA biosynthesis has been determined to be the plasma membrane and the addition of sugars takes place at the reducing ends of the molecule (Laurent and Fraser, 1986). GAG chains are initiated from sugar nucleotide precursors (Silbert, 1982) with the synthesis of a glycoside link which is carried out by the addition of *N*-acetylgalactosamine and glucuronic acid to the chain via their UDP derivatives (Silbert, 1964). The uridine sugar nucleotide precursors undergo a pyrophosphorylase type of reaction (Silbert, 1982) with UDP-glucose and UDP-*N*-acetylglucosamine, which in turn form the "puddling blocks" for polymer synthesis (both types may be formed by a reaction of UTP with a sugar-1-P in the presence of homogenates from proteoglycan-producing tissue). The two forms of nucleotide sugars are the products of the two metabolic pathways which are converted where necessary to forms suitable for inclusion in the various GAGs synthesized (Stockwell, 1979). UDP-glucose can be oxidized to form UDP-glucuronic acid which then may be decarboxylated to form UDP-xylose. Both UDP-glucose and UDP-*N*-acetylglycosamine undergo polymerization at the C-4 position to form UDP-galactose and UDP-*N*-acetylgalactosamine respectively (Silbert, 1982). Termination of CS chain elongation may be achieved by 4-sulphation of the terminal hexosamine residue as this feature along with selective 4- and 6-sulphation, is a property of newly synthesized CS

chains (Otsu *et al.*, 1985). CS chains ending in a *N*-acetylgalactosamine 4-sulphate are unable to accept a glucuronic acid (Rodén *et al.*, 1972). GAG sulphation occurs by the transfer of a sulphate group from PAPS to the sites on the GAG after the incorporation of the sugars (Silbert, 1973). After sulphation has occurred, epimerization of D-glucuronosyl takes place to produce L-iduronic acid in DS (Höök *et al.*, 1974).

Synthesis of the *O*-linked GAGs (all except HA and KS), starts by the xylosylation of serine residues in the core protein. The subsequent actions of xylotransferase, galactotransferases I and II are to form the linkage region, which in turn serves as a primer for the addition of the particular hexosamine and uronic acid in each GAG chain (Sooner and Thompson-Pletscher, 1986). Post-translational modifications of the core protein and the processing of the *O*- and *N*-linked oligosaccharides occurs further in the process when the molecules undergo extensive modifications (Sooner and Thompson-Pletscher, 1986).

Hascall and Hascall, (1981), provide a summary of PG assembly, secretion and aggregation. Synthesis of the core protein is initiated on the ribosomes of the rough endoplasmic reticulum (Fellini *et al.*, 1984). As the protein is being formed, the early stages of assembly of the *N*-linked high mannose-containing oligosaccharides are beginning through a series of highly coordinated enzymatic steps (Kimura *et al.*, 1984) involving a dolichol diphosphate intermediate oligosaccharide. This particular oligosaccharide is transferred intact via a *N*-glycosidic bond to asparagine on the core protein (Stow *et al.*, 1985; Waechter and Lennarz, 1976). Conversion of the high-

mannose oligosaccharide to the sialic acid-containing complex oligosaccharide occurs later in the Golgi (Dunphy *et al.*, 1985). The core protein is then released from the ribosome into the endoplasmic reticulum cisternae, where it travels to the Golgi region of the cell where chondroitin sulphate chains and *O*-linked oligosaccharides are assembled onto the hydroxyls of the corresponding serine, and serine and threonine residues respectively (Thonar *et al.*, 1981). Once the GAG chains have been initiated onto the protein core, the PG is completed very rapidly (Kimura *et al.*, 1981a). PG molecules are secreted into the pericellular environment as soon as they are completed in the Golgi apparatus (Carney and Muir, 1988). The newly formed molecules are collected into secretory vacuoles and over a period of 10-30 minutes are secreted from the cell by exocytosis (Kimura *et al.*, 1979).

Once out in the extracellular matrix, cartilage CSPGs are organized through a highly specific aggregation process involving two additional macromolecules; HA (Hardingham and Muir, 1972) and link protein (Heinegärd and Hascall, 1974). Monomer PGs interact with a long central strand of HA at intervals of approximately 30 nm. The active site of the hyaluronic acid binding region (HABR) of the core protein contains subsites where five repeating disaccharide units may bind (Christner *et al.*, 1979; Hascall and Heinegärd, 1974; Hardingham and Muir, 1973). Furthermore, the active-site exhibits a high affinity for this decasaccharide or larger oligomers of HA, but much lower affinity for shorter oligomers. This interaction is highly specific as no other anionic polymer has been found that will compete with HA for binding and it has a

dissociation constant of about 10^{-6}- 10^{-7} (Christner *et al.*, 1979).

With the addition of the third constituent, the link protein, the PG molecules are essentially locked into place on the HA molecule, with a dissociation constant that is below conventional measurement (Kimura *et al.*, 1979; Hardingham, 1979; Hascall and Heinegärd, 1974). The link protein appears to occur in two forms; a smaller one with a molecular weight of approximately 42,000 and a larger one of about 50,000 molecular weight (Baker and Caterson, 1979; Keiser *et al.*, 1972). The latter appears to contain an additional polypeptide segment as well as some oligosaccharides (Baker and Caterson, 1979). Binding of the link protein occurs along a segment of the HA molecule adjacent to the HABR (Faltz, 1979a; Kimura *et al.*, 1979). The link protein also interacts with a portion of the polypeptide in the HABR (Heinegärd and Hascall, 1979).

The interaction between the PG, HA and link protein produces a relatively stable structure. Although there are small sections of HA that are accessible to enzymes that digest HA, 40 - 50 monosaccharides in the contact areas are not (Faltz, 1979a). The distribution of PGs along the HA molecule appear to be determined by the hydrodynamic volume of the individual monomers, which in turn is determined by the extended lengths of chondroitin sulphate chains that radiate from the core protein (Hascall and Hascall, 1981).

Although it is apparent that assembly of the PG aggregates occurs some time after secretion, the mechanisms for aggregation and organization of the aggregates within the collagen network are not known. It has been suggested that aggregation of PGs is critical

for immobilizing them in the tissue, as the non-bound monomers may diffuse out of the matrix (Hascall and Hascall, 1981). Although PGs are able to aggregate as soon as they are synthesized, there is some evidence that a maturation step is required to convert the PG from an inactive to a fully active aggregating form (Hardingham *et al.*, 1984). In addition, not all PGs are secreted into the matrix , since some complexes remain attached to the lipid bilayer. These types of PGs usually contain a small number of GAG chains (Höök and Kjellén, 1984).

For the most part, the complex regulation of PG biosynthesis remains unknown. There have been some molecules linked to stimulation of the PG synthetic process such as; insulin (Purvis *et al.*, 1984), insulin-like growth factor I (Jennings *et al.*, 1980), ascorbic acid (Prins *et al.*, 1982), platelet-derived growth factor (Prins *et al.*, 1982), and pituitary-derived growth factor (Kato and Gospodarowicz, 1985a). Interestingly, high doses of glucocorticoids have a degradative effect on the cartilage matrix, while low doses of both hydrocortisone and dexamethasone have been found to have a stimulatory effect (Kato and Gospodarowicz, 1985b). In contrast, prostaglandins such as E_1 , E_2 and A_1 have an inhibitory effect on cartilage PG synthesis (Fransson *et al.*, 1983; Mitrovic *et al.*, 1981; Lippiello *et al.*, 1978; Malemud and Sokoloff, 1977).

The degradation of PGs has been viewed as the opposite of the synthetic process, (Rodén, 1980) while the mechanism of PG turnover remains unresolved. The catabolic pathway of the GAG moiety is not clear. Although there is evidence of GAG degradation, the extent is not known as studies have found intact GAGs in the urine

(Silbert, 1982). The enzymatic degradation of PGs and GAGs was first noted as a spreading factor (Silbert, 1982). In vitro enzyme studies were correlated with the in vivo situation to suggest that enzymes similar to the reported spreading factor were also present in GAG breakdown in vivo. Reflecting upon the structure of PG aggregates, three groups of enzymes are involved in degradation. These groups are the proteinases and the glycosidases (Stockwell, 1979) as well as the sulphatases. In a sequential fashion, beginning at the non-reducing end of the molecule, the enzyme groups remove the glycosyl as well as the sulphate groups (Rodén, 1980). The most familiar GAG degrading enzyme is hyaluronidase (Rodén, 1980; Silbert, 1978).

Proteinases are known to break down the core protein structure of PGs resulting in reduced functionality of the molecules (Hascall and Hascall, 1981). The breakdown products consist of GAG chains and smaller peptide fragments with fewer chains which do not have the large hydrodynamic domains of the intact PGs. Trauma or inflammation may release proteinases into the matrix destroying PGs and initiating the repair processes (Hascall and Hascall, 1981).

3.3. Functional Aspects of PGs and GAGs

Proteoglycans are highly branched in solution and occupy very large hydrodynamic volumes relative to their molecular weights (Reihanian *et al.*, 1979; Pasternack et al., 1974; Hascall and Sajdera, 1970). Under compressive load, the molecules are reversibly compressed with solvent being expressed from the molecular domain. As this occurs, the intramolecular interactions between the relatively stiff and

polyanionic GAG chains also increase. Once the load is removed, the molecular domains expand to their original size. This hydrodynamic property is critical to the normal load-bearing function of cartilages on the surfaces of long bones (Hascall and Hascall, 1981). Cartilage matrix is a composite structure, with the collagen fibrils providing a network that determines tissue shape and resists tensile forces, and the interspersed PG aggregates creating a viscous gel that absorbs compressive forces.

The concentration of PGs in load-bearing hyaline cartilages is three to five times more than would be possible if the molecules were fully extended (Hascall and Hascall, 1981). The effect of restricting their molecular domain to about 20% of their maximum increases the charge density within the domains considerably. The end result is a molecular configuration that is able to resist an equivalent load with less total deformation, much as a compressed spring would. Furthermore, the competition between PG molecules for available solvent also restricts the domains of the molecules (Hascall and Hascall, 1981). Therefore, it appears that the ability of a particular region of hyaline cartilage to resist compressive loads is dependent upon the capacity of the resident chondrocytes to synthesize and secrete PGs into the highly concentrated adjacent extracellular matrix.

In general, it appears that GAGs are capable of influencing processes such as maintenance of tissue structure, collagen fibrillogenesis, regulation of water flow and water retention, regulation of metabolite concentration and transport, and protection against high tissue pressures (Laurent *et al.*, 1969).

In older cartilage or cartilage that has undergone considerable wear and tear, it

appears that the chondrocytes are unable to replace proteoglycans sufficiently (McDevitt *et al.*, 1981). Under these conditions, the PGs that are synthesized are less effective structures, such as molecules with fewer and shorter chondroitin sulphate chains. The function of the hyaline cartilage is impaired possibly leading to osteoarthritis (Hascall and Hascall, 1981). The mechanism of cellular regulation of PG synthesis is not understood, although local environmental factors seem to play a role.

3.4. Proteoglycans and Glycosaminoglycans in Connective Tissue

The extracellular matrix of connective tissue is comprised of two major elements (Hunziker and Schenk, 1987); the first is a variety of insoluble fibers and the second is a homogenous transparent layer of amorphous ground substance. PG aggregates are responsible for approximately 95% of the dry weight of the ground substance. This predominance of PGs is achieved in spite of the high concentration of PGs in an underhydrated state (approximately 20 to 40 mg/ml) (Hunziker and Schenk, 1987). This feature of PGs poses major problems for aqueous chemical fixation of PGs.

Collagen and PGs work together to provide articular cartilage the unique feature of reversible deformity (McDevitt, 1973). PGs of cartilage exist as several subpopulations that differ in size and composition. The majority of PGs are found as large aggregates (McDevitt, 1973) that form an amorphous gel (Sweet *et al.*, 1977). Nonaggregating PGs make up a small portion of the total PG content (Myers and Mow, 1983; Muir, 1980). These compounds are always found bound to protein in hyaline cartilage (McDevitt, 1973). PGs of cartilage have been found to be distributed mainly

in the central region where about 90% of GAGs aggregate with HA (Maroudas, 1979). The GAGS of cartilage are C_4S, C_6S, KS and HA (Carney and Muir, 1988; Stockwell, 1979; McDevitt, 1973), although considerable variation between different regions of cartilage have been reported (Silbert, 1978). In hyaline cartilage, the most abundant PG is a high aggregating species containing both CS and KS chains, although the KS content is more variable and increases with age (Hardingham *et al.*, 1986).

It appears that GAG distribution and content varies with the depth from the surface (Bayliss *et al.*, 1983; Lemperg *et al.*, 1974; Stockwell, 1967), and topographically in different articulating or non-articulating areas (Bjelle, 1974). GAGs account for 5 - 15% of the dry weight of cartilage. CS is the main constituent (50%), while KS varies from 4 to 20% of the total, and lastly HA usually represents about 1% (Silbert, 1978). The low percentage of HA can be explained by the aggregation of CSPG. In articulating areas, there is more HA and much more KS than in areas of minimal contact (Sweet *et al.*, 1977). CS is described as territorial, while KS is predominantly inter-territorial, except in the deeper zones of aging tissues where KS is found close to the chondrocytes (Hardingham *et al.*, 1986). Normally, an increase of KS is observed in the deeper layers of cartilage while PG complexes are increased in the middle zone as compared to the articular surface (Ratcliffe *et al.*, 1984). Furthermore, the type, concentration, and distribution of PG complexes in connective tissue may reflect the load regimen that the tissue is exposed to *in vivo* (Scott *et al.*, 1989).

3.4.1. Proteoglycans of the TMJ Articular Disc

The composition of the extracellular matrix in articular discs appears to consist of sheets of collagen fibrils and fibers dispersed in a sparse interfibrillary matrix, except in the areas immediately adjacent to the cells (Granström and Linde, 1973) where PG complexes and associated filaments are found (Ghadially, 1978). In the central zone of the disc, the inter-territorial matrix is mostly dense fibrous tissue (Silva, 1969). This composite structure has a fibrochondrocytic appearance and features a considerable degree of flexibility (Silva, 1969).

Many differences exist between tissues that are primarily composed of fibrocartilage versus those of a mostly cartilage composition. For example, approximately 50% of the total amount of GAGs of the nucleus pulposis of the vertebral column is KS, while the remainder is mainly CS (Hallen, 1958). Habuchi *et al.* (1973) suggest that the meniscal GAGs represent a separate family which is specifically adapted to the function of fibrocartilages. Interarticular disc PGs have been found to have a smaller size and more KS chains than articular cartilage PGs, although they have the same capability of aggregation (Muir, 1981). In general, PGs are minor components of fibrous connective tissues comprising less than 1% of the dry weight of the tissue (Vogel and Heinegärd, 1985).

Topographical descriptions of the GAGs of the articular disc indicate that the central load-bearing zone is marked with GAGs. Öberg (1964) reported an increase in the uptake of [^{35}S] in guinea pigs in the central part of the disc, while Kopp (1976) used histochemical methods to find a high proportion of sulphated GAGs in this zone.

Furthermore, the sulphated GAGs in this area displayed characteristics similar to CS, DS and KS (Kopp, 1976). By contrast, Mills *et al.* (1988) observed a higher distribution of GAGs in the anterior and posterior bands of the rabbit craniomandibular articular disc, with distinct populations of C_6S and KS. Quantitative analysis of GAGs extracted from pooled bovine articular discs also show predominant amounts of CS and DS with smaller proportions of HA and KS (Nakano and Scott, 1989). Another quantitative study by Granström and Linde (1973) suggested that DS and HA are the two major GAGs of the articular disc. DS was thought to be the largest fraction in the rabbit, dog and monkey articular discs, while HA was found to be the major fraction of the rat disc. However, Granström and Linde recognized that their method did not specifically measure DS content as other GAGs such as C_4S, C_6S exhibited similar electrophoretic mobility as DS. Analysis of the PG content of the rabbit knee meniscus indicated that it consists of C_6S (72%), C_4S (19%) and DS (5%) (Webber *et al.*, 1984).

The variation of PG content in different tissues of various animals may be due to a multitude of factors including; differences in the weight bearing of the articulations, simple species differences (Webber *et al.*, 1984), as well as differences in diet. Although the relationship between GAG variation and function of a particular connective tissue is unclear, the proportion of large and small PGs in a tissue may be an important indicator of their roles in producing and maintaining a functional matrix (Vogel and Heinegärd, 1985).

3.4.2. Hyaluronic Acid and the TMJ Synovial Fluid

Synovial Type A cells synthesize HA which is exported into the synovial fluid. The concentration of HA in synovial fluid is approximately 3.3 mg/ml. Because these polyionic molecules attract and bind a tremendous volume of water, this concentration is approximately ten times that required to entirely fill its volume in solvent[4]. Therefore, HA molecules in synovial fluid are extremely concentrated and overlapping each other, trapping large amounts of water and various ions (Balazs, 1982). The size, shape, and volume of HA molecules and their interaction with water and free ions are expressed as the limiting viscosity number of HA. This number has been found to be significantly reduced in arthritic conditions (Balazs, 1982, 1969).

The distribution of HA in the TMJ is not uniform. The areas of lowest concentration are the joint spaces, whereas the highest concentrations are found on the surfaces of articular cartilage and the articular disc. In the abundant areas, HA forms a layer several microns thick and penetrates into these tissues to a depth of 50 to 100 μm (Balazs, 1982, 1971, 1966). This HA layer serves many purposes. Firstly, it allows free diffusion of small nutrients, ions, and water into and out of the articular cartilage, disc and other tissues to provide nutrition and removal of metabolic wastes in these avascular structures. Secondly, it serves as a barrier to the leakage of large molecules, such as proteoglycans from the articular cartilage. Lastly, it serves as a lubricant.

Biological lubrication includes protection of cells from compressive loads, shear

---

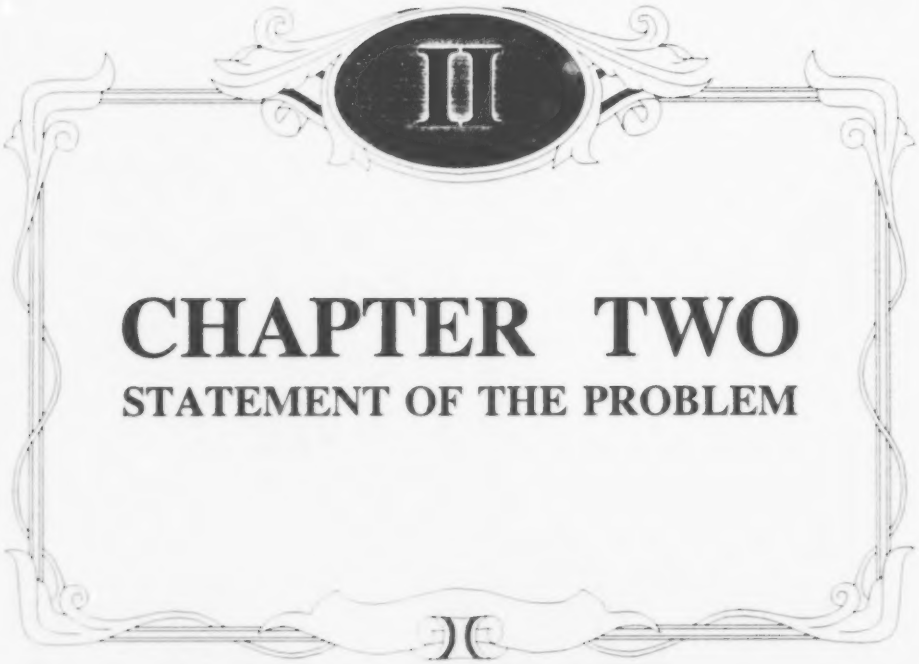
[4] HA occupies a spheroidal domain of 0.3 μm.

stress, surface contact, and the dissipation of energy. Balazs (1982, 1970, 1969, 1968, 1967) reports that HA exhibits both viscous and elastic properties, depending on the magnitude of strain frequency applied to the molecule. At low strain frequencies, normal synovial fluids behave as viscous fluids, whereas at high strain frequencies, which occur with high impact loading, they behave as elastic solids. During normal joint movement without excessive pressure, low strain frequencies are applied to the HA molecules, which then distort and slide over one another, resulting in viscous flow. This activity occurs only at the level of the HA molecules; the collagen fibers on the surface of the disc remain apart and hence do not abrade each other. However, during high strain frequencies (high impact loading), the molecules are unable to adjust configurationally and become entangled and deform sinusoidally, thereby forming elastic solids, which store potential energy in their interstices.[5] As stress is released, energy is gradually released as heat and the elastic solid slowly rebounds, once again establishing a viscous flow. Under high strain frequencies, the elastic solid formed by the HA molecules absorbs the mechanical energy while separating the collagen surfaces thus preventing their deterioration. These rheological changes from viscous fluid to elastic solid occur without degradation of HA.

In traumatic, degenerative, or inflammatory arthritis, the HA molecules are significantly reduced in size with a consequent reduction in viscosity (Balazs 1982, 1967). As a result, it acts merely as a viscous fluid and is unable to protect the collagen

[5] This release of energy may contribute to an audible "pop".

surfaces under high strain frequency. This initiates a cycle of increased surface damage, increased joint inflammation, and decreased HA viscosity, progressing to joint deterioration. Therefore, normal synovial fluid viscosity is essential to normal TMJ surface protection.



CHAPTER TWO

STATEMENT OF THE PROBLEM

STATEMENT OF THE PROBLEM

Temporomandibular joint (TMJ) dysfunction is a clinical problem that has attracted much attention in recent years. Practitioners take special interest in this problem, whether they desire to or not, because, while they are often praised for management of the dysfunction, they are also accused of contributing to the problem.

Despite significant volumes of research performed in this field over the last decade, research efforts have been unable to settle controversy surrounding this topic. This situation has led Greene[1] (1988) to state that "the TMJ is the single most controversial area of dentistry and in no other area of modern dentistry have so many agreed to disagree". To further understand this dilemma, one must consider the quality of this research. A review of literature on the subject of temporomandibular joint dysfunction discloses an excessive representation of viewpoint publications and case reports in comparison with the number of controlled studies. While these viewpoint articles and case reports offer little or no contribution to our understanding of TMJ dysfunction, they often describe opinions that are various and conflicting. Perhaps this situation is a reflection of the current lack of knowledge on this issue.

In the category of non-inflammatory derangements of the TMJ, the articular disc is mainly responsible for the signs and symptoms in the joint (Solberg, 1986; Bell, 1990). Although it is generally accepted that mechanical articulating forces are placed

[1] Greene (1988) in his article "Orthodontics and Temporomandibular Disorders"

directly on the temporomandibular disc (Copray *et al.*, 1985; Hinton, 1981; Blackwood, 1966), characteristics of these forces such as intensity, duration, direction, and effect on tissues are not established. Furthermore, it is not known which forces are physiologic (Drachman and Sokoloff, 1966) and which ones are pathologic (Bell, 1990; Moffett, 1984a). In addition, the basic mechanical properties of the disc and the biological activities that determine these properties are poorly understood.

At present the general consensus is that a degenerative response of the articular tissues occurs when the *adaptive ability* (functional range) of these tissues is exceeded. Joint tissues sustain a level of function to develop the specific properties required to maintain that function. When tissues are unable to develop the characteristics necessary to manage the stresses placed upon them, these tissues may be damaged and may lead to dysfunction of the joint.

Factors associated in predisposing or leading to joint dysfunction are numerous with many being very controversial. Unfortunately some of this controversy has spilled over from an academic forum to a media and/or legal debate (Pollack, 1988). Hopefully, in the near future, medical, dental, and legal practitioners alike will all realize that clinical problems can only be settled by scientific inquiry and controlled clinical trials and in no other manner[2].

Although current research is weighted towards the clinical management of

---

[2] According to Huber (1991), in his article entitled "Junk Science in the Courtroom", the legal profession is developing an awareness of the potential absurdity of some "expert" testimony.

temporomandibular joint dysfunction, increased emphasis should be applied towards prevention and/or early diagnosis of the problem. This relies upon a knowledge of the etiology and pathogenesis of the dysfunction, which in turn depends upon a thorough understanding of normal growth and development of the temporomandibular joint.

Biochemical methods may be utilized to study growth and development of the TMJ articular disc as well as pathogenesis of TMJ dysfunction. The biochemical content of the articular disc may provide information relating to growth and development of the disc, function of the disc, and the ability of the discal tissues to resist deformation and perforation. **To date, there have been no biochemical studies of the fetal human temporomandibular joint articular disc presented in the literature.** It is essential to understand the biochemistry of the TMJ articular disc during normal growth and development before one may even begin to understand the biochemical changes that occur in the disc during TMJ dysfunction. It is in this light that this project may offer a contribution.

Therefore the purposes of this study are:

1. To study the macroscopic and microscopic structure of the fetal human TMJ articular disc.
2. To determine which glycosaminoglycan and proteoglycans are present in histologic sections of the fetal human TMJ articular disc.
3. To study the synthesis of PGs and GAGs of fibroblasts cultured from the fetal human articular disc.
4. To establish relative quantities of PGs and GAGs produced by cells of the

articular disc *in vitro*.

5. To establish baseline data for further study of the TMJ articular disc.

CHAPTER THREE

MATERIALS AND METHODS

MATERIALS AND METHODS

OUTLINE

1. Materials 81

1.0. Introduction 81

1.1. Fetal Human TMJ Articular Discs 82

1.1.1. Medical History of the Fetal Specimens 83

1.2. Histology and Immunohistochemistry 84

1.2.1. Histology 84

1.2.2. Immunohistochemistry 84

1.3. Cell Culture 85

1.4. Isolation and Preparation of PGs and GAGs 86

1.5. Enzyme Linked Immunosorbent Assays 87

1.6. Uronic Acid Analysis 88

1.7. Cellulose Acetate Electrophoresis 88

1.8. Di Ferrante Method for Iduronic Acid 89

1.9. Thin Layer Chromatography 89

1.10. Polyacrylamide Gel Electrophoresis 89

1.11. Immunoblotting 90

2. Methods 90

2.1. Removal of the Articular Discs 90

2.2. Histology and Immunohistochemistry 92

2.2.1. Histology 92

2.2.2. Immunohistochemistry 93

2.3. Cell Culture 95

2.3.1. Passage of Cells 97

2.3.2. Freezing and Storage of Cells 98

2.3.3. Collection of Conditioned Medium 99

2.4. Isolation and Preparation of PGs and GAGs 99

2.4.1. DEAE-Sephacel Column 99

2.4.2. DMB Assay 100

2.4.3. Dialysis and Lyophilization 100

2.4.4. Enzymatic Degradation of PGs and GAGs 100

2.4.4.1. Chondroitinase ABC 100

2.4.4.2. Chondroitinase ACII 101

2.4.4.3. Chondroitinase B 101

2.4.4.4. Streptomyces Hyaluronidase 102

2.4.4.5. Papain 102

2.5. Enzyme Linked Immunosorbent Assays 103

2.6. Uronic Acid Analysis 104

2.7. Cellulose Acetate Electrophoresis 105

2.8. Di Ferrante Method for Iduronic Acid 106

2.9. Thin Layer Chromatography 107

2.10. Polyacrylamide Gel Electrophoresis 107

2.11. Immunoblotting 108

FLOWCHART OF EXPERIMENTAL METHODS

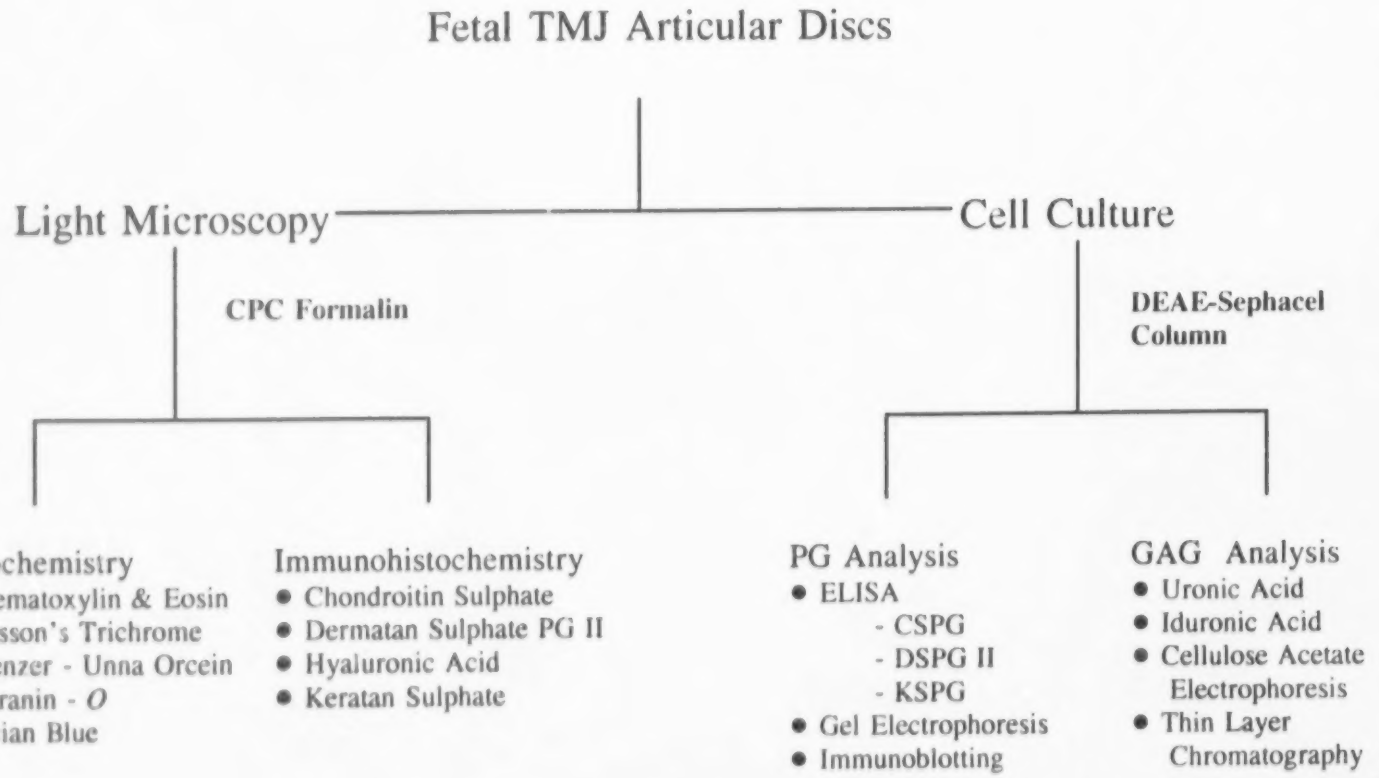


Figure III.1.0.A.
Flowchart of Experimental Methods

1. Materials

1.0. Introduction

Knowledge of TMJ structure, function, and derangements has been obtained from species of animals such as; bovine calves (Nakano and Scott, 1989; Scott *et al.*, 1989) chicks (Murray, 1969; Drachman and Sokoloff, 1966), guinea pigs (Silva, 1969), mice (Kantomaa *et al.*, 1988; Glasstone, 1971; Frommer, 1964; Levy, 1949), monkeys (McNamara *et al.*, 1975, 1974; McNamara 1973; Zimmerman, 1971; Cutler, 1968), rabbits (Kantomaa, 1984a, 1984b; Barnett *et al.*, 1963); and rats (Carvalho, 1990; Lekkas *et al.*, 1988; Gazit *et al.*, 1987; Petrovic *et al.*, 1985, 1975; Koski and Ronning, 1982, 1969; Buchner, 1982; Katz *et al.*, 1979; Appleton, 1975; Furstman, 1966; Frommer, 1966; Cunat *et al.*, 1956; Cabrini *et al.*, 1941). Study of human material includes that of the human fetus (Morimoto *et al.*, 1987; Perry *et al.*, 1985; Wong *et al.*, 1985; Van Dongen, 1970; Yuodelis, 1966; Furstman, 1963) and that of cadaveric material (Hellsing *et al.*, 1985; Hansson *et al.*, 1976; Carlsson *et al.*, 1974; Öberg, 1971; Coleman *et al.*, 1955). In addition to these human studies are the clinical studies that are too numerous to mention.

Animal models are useful and often are the only choice in biological studies. As well, they allow the experimenter to control many variables. Although much has been learned from animal studies, the results must always be extrapolated to the human situation. Investigators such as McNamara (1975) and Petrovic *et al.* (1975) suggest a

strong relationship between experimental studies of monkeys and rats compared to those of humans. However, the strength of the relationship probably depends on the nature and design of the experiment. Nevertheless, there always remains doubt concerning the relevance of such comparisons.

When human material is utilized, the burden of such comparisons is lifted. However, other problems arise such as; the inability to select the age of the specimen, limited availability of study material, and ensuring that the samples are representative of the normal situation.

The present study makes use of several experimental methods to investigate the research problem. Although some methods are limited by the amount of material available, the selection of methods was based on accuracy, feasibility and ability to relate to data obtained from other methods. Refer to Appendix I for a flowchart of experimental methods.

1.1. Fetal Human TMJ Articular Discs

Human fetal material was obtained from the University of Alberta Hospital in Edmonton, Alberta. Selection criteria of the fetuses were as follows:

1. Essentially "normal" in development[1]
2. No genetic disorders[1]
3. No pathologies that may influence growth and development of the TMJ[1]

[1] Determined by Medical Pathologist

4. Within one week post-mortem[2]

5. Not placed in any fixative[2]

1.1.1. Medical History of the Fetal Specimens

The TMJ articular discs from two fetuses were obtained for this study. Both met the selection criteria mentioned above. Their medical information is summarized in Table III.1.1.1.A.

Date of Death	Date of Autopsy	Chart Number	Sex	Intrauterine Age
Nov. 30,1990	Dec. 4, 1990	PM 90-314†	Male	25 weeks
Jan. 2, 1991	Jan. 7, 1991	9919002‡	Male	20-22 weeks

Table III.1.1.1.A.
Medical History of the Fetal Specimens

† For the remainder of this study, the articular discs from this specimen will be referred to as Fetal Disc 1 **(FD1)**.

‡ For the remainder of this study, the articular discs from this specimen will be referred to as Fetal Disc 2 **(FD2)**.

[2] To allow culturing of viable cells

1.2. Histology and Immunohistochemistry

1.2.1. Histology

Formalin and glass histologic slides were obtained from Fisher® Scientific, Edmonton, Alberta, Can., while cetylpyridinium chloride was obtained from Sigma® Chemical Co. St. Louis, MO., USA. Alcian Blue 8GX was purchased from Terochem Labs Ltd., Edmonton, Alberta, Can. Chondroitinase ACII was from Seikagaku America® Inc., Rockville, MD., USA., while *Streptomyces* hyaluronidase was from Calbiochem®, San Diego, CA., USA.

1.2.2. Immunohistochemistry

The monoclonal antibody to bovine skin DSPG II/PDS (6D6) was prepared and characterized by Pringle *et al.* (1985). Anti-CS (clone CS-56) and anti-KS monoclonal antibodies were purchased from ICN®, Lisle, IL., USA. The anti-CS monoclonal antibody (CS-56) was produced according to the criteria set by Avnur and Geiger (1984), while the anti-KS antibody (5D4) was prepared as described by Caterson *et al.* (1983). The antibody to HA (NDOG1) was manufactured by Serotec® Ltd. using the methodology of Sunderland *et al.* (1985) and purchased through Bioproducts For Science®, Inc., Indianapolis, IN, USA.

Normal goat serum containing 0.2% (w/v) NaN_3 was obtained from GIBCO®, Burlington, Ontario, Can. The goat anti-mouse IgG $F(Ab')_2$ fragments (heavy and light chains specific) (Lot# 26760) were manufactured by Cappel® Ltd. and purchased through

Organon Teknika® Corp., West Chester, PA., USA. Peroxidase-antiperoxidase complex (Clono-PAP®) of mouse monoclonal origin (Lot #MC10) was obtained from Sternberger-Meyer Immunocytochemicals® Inc., Jarrettsville, MD., USA. Diaminobenzidine tetrahydrochloride was from Sigma® Chemical Co., St. Louis, MO., USA.

The enzyme Chondroitinase ABC was obtained from Seikagaku America® Inc., Rockville, MD., USA., while *Streptomyces* hyaluronidase was from Calbiochem®, San Diego, CA., USA.

1.3. Cell Culture

Dulbeco's modified Eagles medium (DMEM), fetal calf serum (FCS), antibiotics and antimycotics[3], and NUNC® cryotubes were purchased from GIBCO® Canada, Burlington, Ontario. Petri dishes (7.5 cm^2), disposable tissue culture flasks (75 cm^2 and 150 cm^2), and disposable plastic centrifuge tubes (15 ml and 50 ml) were obtained from Corning® Canada, Richmond Hill, Ontario. Sterile borosilicate pipets (1,2,5, and 10 ml) were from Fisher Scientific®, Edmonton, Alberta, Can. Medigas® Alberta supplied a Forma Scientific® model 3029 CO_2 incubator, compressed O_2 and CO_2 as well as Union Carbide® LRS 19 and XR12 liquid nitrogen refrigerators. Chemicals such as 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES®) and dimethylsulfoxide (DMSO) were purchased from Sigma® Chemical Co., St. Louis, MO, USA. Sterivex®-GS filters

[3] An antibiotic and antimycotic mixture (25 ml), containing: 10 000 units/ml penicillin 6 sodium, 10 000 mg/ml streptomycin sulfate and 25 mg/ml amphotericin B as fungizone (0.85%) was added to every 2 litres of medium.

(0.22 μm) and accompanying peristaltic pump and Millex®-GS filters (0.22 μm) were obtained from Millipore® Ltd, Mississauga, Ontario, Can. Alkyldimethylbenzyl ammonium chloride (Roccal®), used as an antibacterial agent in the incubator water supply (10 ml/5 l H_2O), pipet wrap, autoclavable garbage bags, autoclave tape, adsorbent cotton, haemocytometer and Fyrite® gas analyzer were from Canlab®, Edmonton, Alberta. A Wild Leitz® Diavert inverted stage light microscope equipped with a Wild Photoautomat® MPS 45 and 35 mm camera was used to examine and photograph the cell cultures.

1.4. Isolation and Preparation of PGs and GAGs

DEAE-Sephacel was obtained from Pharmacia® Canada Ltd, Dorval, Quebec, Canada. Urea (reagent grade), NaCl, and ethylenediamine tetraacetate (EDTA) were from Fisher Scientific®, Edmonton, Alberta, Can., while Tris-HCl, NaN_3, benzamidine hydrochloride, phenylmethanesulphonyl fluoride (PMSF) and pepstatin were from Sigma® Chemical Co. St. Louis, MO., USA. Leupeptin was from IAF Biochem® International Inc., Laval, Quebec, Can. *N*-ethylmaleimide (NEM) was from Aldrich® Chemical Co.

Each collection of DMEM cell culture medium was given a nomenclature (Table III.1.4.A.). Details of the collections are also listed.

Column #	Source & Passage	FCS Content (%)	Volume (ml)
DEAE1	FD1-P3	2	220
DEAE2	FD2-P2	2	220
DEAE3	FD2-P2	0	220
DEAE4	Unconditioned	2	220
DEAE5	FD2-P2	2	555
DEAE6	FD1-P3	2	465

Table III.1.4.A.
Description of Cell Culture Medium

Rolls of Seprapore® 10 000 - 12 000 molecular weight cut-off dialysis tubing were purchased from Fisher Scientific®, Edmonton, Alberta, Can.

Chondroitinase ABC (from *Proteus vulgaris*), chondroitinase B (from *Flavobacterium heparinum*) and chondroitinase ACII were from Seikagaku America® Inc., Rockville, MD., USA. Papain (twice crystallized) was purchased from Sigma® Chemical Co., St. Louis, MO., USA. *Streptomyces* hyaluronidase was from Calbiochem®, San Diego, CA., USA.

Water was distilled tap water redistilled with a Corning® Megapure distillation unit and/or deionized and sterilized using a Milli-Q® system equipped with a 0.22 μm Millistak®-GS filter (Millipore Ltd.).

1.5. Enzyme Linked Immunosorbent Assays

Costar®, Cambridge, MA., USA provided flat bottom high binding EIA/RIA

plates. Polyoxyethylene sorbitan monolaurate (Tween®) was obtained through Fisher Scientific®, Edmonton, Alberta, Can. Sigma® Chemical Co., St. Louis, MO., USA supplied bovine serum albumin (BSA) and *p*-nitrophenylphosphate. The Titertek® Multiskan and interference filters were purchased from Flow Laboratories® Ltd.

The CSPG and DSPG II used to coat the plates as well as antigenic standards were previously isolated from adult bovine TMJ articular disc and adult bovine skin, respectively, in Dr. P.G. Scott's laboratory.

The secondary antibody was an alkaline-phosphatase-conjugated goat anti-mouse Ig (G+A+M) antibody from Zymed®, San Francisco, CA., USA.

1.6.Uronic Acid Analysis

The 16 x 100 mm disposable glass test tubes were purchased from Fisher Scientific®, Edmonton, Alberta. The D-glucurono-γ-lactone and L-iduronolactone (from hog skin) standards were purchased from Sigma® Chemical Co., St. Louis, MO., USA.

1.7. Cellulose Acetate Electrophoresis

Sepraphore® III cellulose polyacetate electrophoresis strips (2.5 cm x 15.2 cm), Sepra Clear® II and the electrophoresis tray were obtained from Gelman Sciences®, Ann Arbor, Mich., USA. Alcian Blue (8GX) was purchased from Terochem Labs Ltd., Edmonton , Alberta, Can.

1.8. Di Ferrante Method

Basic fuchsin (C.I. 42510) was from Serva Feinbiochemica®, New York, NY., USA. Sodium-metabisulfate, activated decolorizing carbon and the disposable glass test tubes (10 x 120 ml) were purchased from Fisher Scientific®, Edmonton, Alberta. $NaIO_4$ was from J.T. Baker Chemical Co., Phillipsberg, NY., USA. $NaClO_4$ was supplied by G. Frederick Smith Chemical Co., Columbus, OH., USA. H_2SO_4 was from BDH® Chemicals.

1.9. Thin Layer Chromatography

The standard unsaturated disaccharides (ΔDi-0S, ΔDi-4S, and ΔDi-6S) were manufactured by Seikagaku Kogyo Co., Tokyo, Japan and were obtained through Miles Laboratories, Elkhart, Ind., USA. The HA standard was from Sigma® Chemical Co., St. Louis, MO., USA. Silica gel plates of Kieselgel® 60 F_{254} (20 x 20 cm) were manufactured by Merck & Co. and were purchased through Terochem Labs Ltd., Edmonton, Alberta, Can.

1.10. Polyacrylamide Gel Electrophoresis

Acrylamide, sodium dodecyl sulphate (SDS) ammonium persulphate, *N,N'*-methylene-bis-acrylamide, *N,N,N',N'*-tetramethylethylenediamine (TEMED®), the high and low molecular weight standards, Coomassie Blue R250, Bromophenol Blue, the Trans-Blot® Cell, the Mini-Protean® II electrophoresis cell and the BioRad® 400 power supply were from BioRad® Labs. Canada Ltd., Mississauga, Ontario, Can.

Gels were dried on a GSD-4 Gel Slab Drier (Pharmacia® Canada Ltd., Dorval, Quebec, Can.) connected to a lyophilizer.

1.11. Immunoblotting

Nitrocellulose membranes (Schleicher and Schuell) were purchased through Chemonics Scientific Ltd., Edmonton, Alberta, Can. Bovine serum albumin (BSA) and 3,3'-diaminobenzidine tetrahydrochloride were purchased from Sigma® Chemical Co., St. Louis, MO., USA. Pyronin Y was from Canlab®.

2. Methods

2.1.1. Removal of the Articular Discs

The most obvious access to the TMJ articular disc is through an external incision made slightly anterior to the tragus. However, a guideline was imposed that ruled out this approach. Therefore, another path of entry was necessary. A further consideration was the difficulty in locating and removing a small structure such as the articular disc without damaging it in the process. In view of these difficulties, it was decided to remove the TMJ *en bloc* in the morgue and continue the dissection under a dissection microscope. The approach that was implemented is a modification of the method described by Wright and Moffett (1968). They describe a technique to remove the TMJ *en bloc* by applying a trephine (plug cutter) to the floor of the middle cranial fossa.

Since there were no trephines available in the autopsy room, the modification was the use of the small square end of an autopsy saw to cut through the temporal bone from the floor of the middle cranial fossa[4]. The bone on the superior aspect of the articular fossa was landmarked and saw cuts roughly in the shape of a rectangle were made through the bone to free this area from the remainder of the temporal bone. Once the bone of the articular fossa was free, it was possible to push up on the gonial angle of the mandible to push the TMJ and the neck of the condyle into the cranial fossa. The soft tissues were dissected away to expose the neck of the condyle. Surprisingly, the condylar neck was thin enough to allow the condylar head to be cut away from the rest of the mandible with scissors.

The tissues were then immediately transferred to a dissecting microscope where the articular discs were carefully isolated from the capsule and tissues of the posterior attachment using fine scissors, a dissecting pick, and a #15 (Bard Parker) scalpel blade. The articular discs were then rinsed with water to remove synovial fluid and photographed (Plate III.2.1.1.A.). Note that the articular disc is approximately 5 mm in length at this age.

Following removal of the TMJ articular discs, one side was placed in fixative for histologic study while the other was reserved for cell culture.

---

[4] At autopsy the calvarium is detached in order to remove the brain for a brain weight measurement.

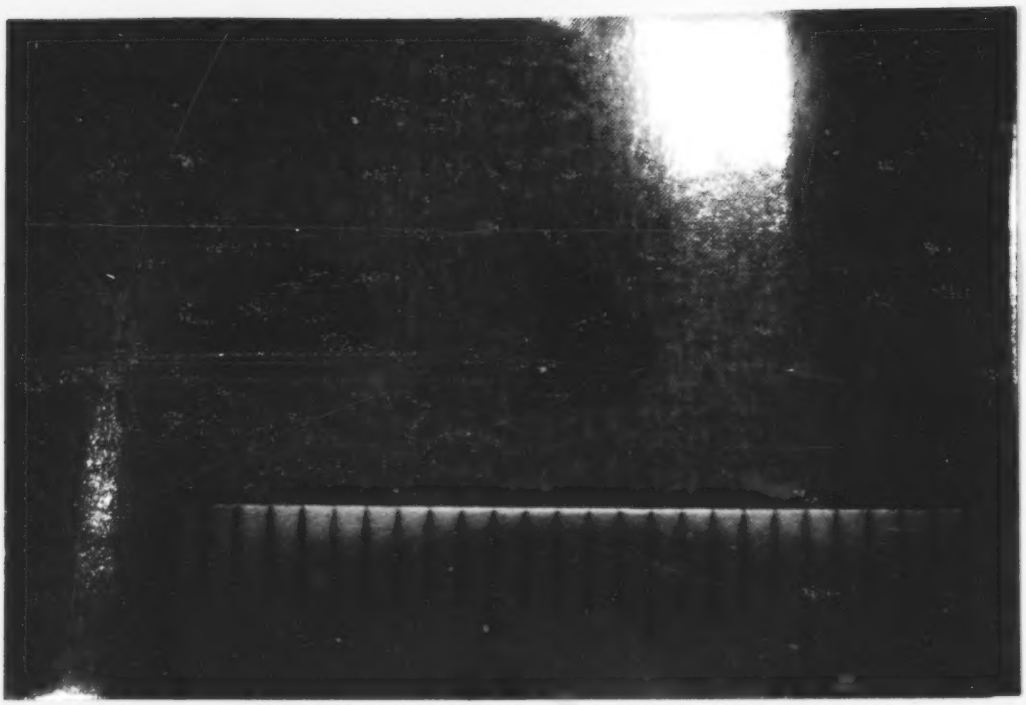


Plate III.2.1.1.A.
TMJ Articular Disc from a Human Fetus

2.2. Histology and Immunohistochemistry

The articular discs were fixed in 4% formalin containing 0.5% cetylpyridinium chloride (Williams and Jackson, 1956). Following fixation, the discs were dehydrated and embedded in paraffin wax (Drury and Wallington,1967). They were then sectioned sagitally at a thickness of 5 μm, and mounted on glass slides .

2.2.1. Histology

The sections were stained with: Haematoxylin and Eosin, Safranin-*O*

(Rosenberg,1971), Masson's Trichrome (Masson, 1929), Taenzer-Unna orcein and Alcian Blue (Lev and Spicer, 1964).

Following a method described by Brinkley and Morris-Wiman (1987b), Alcian Blue was used to stain tissues following digestion with Chondroitinase ACII or *Streptomyces* hyaluronidase. For the digestion methodology refer to Section 2.2.4. Enzymatic Degradation of Proteoglycans. The peak spectral absorbance of the tissue sections were read at 600 nm on a Gilford® 252 spectrophotometer with a linear transporter.

2.2.2. Immunohistochemistry

Sections were stained immunochemically following the procedure described by Sternberger (1979) with monoclonal antibodies to; chondroitin sulphate (Avnur and Geiger, 1985) , dermatan sulphate proteoglycan II (DSPGII/PDS) (Pringle *et al.*, 1985), keratan sulphate (Caterson *et al.*, 1983), and hyaluronic acid (Sunderland *et al.*, 1985) (Table III.2.2.2A.).

Ab Name	Target Ag	Ab Class	Production	Dilution	Diluent
6D6	PDS	IgG	Supernatant	1:5	TBS
CS56	CS	IgM	Ascites	1:1000	PBS
NDOG1	HA	IgM	Supernatant	1:4	PBS
5D4	KSPG	IgG	Ascites	1:1000	TBS

Table III.2.2.2.A.
Antibodies for Immunohistochemistry

The paraffin embedded sections were warmed slightly over a hot plate for a few seconds to attach them securely to the slides. Dewaxing of the slides was performed by rinsing in 4 successive changes of Hemo-D®[5] for 1 minute each, followed by 4 successive changes of absolute ethanol for 1 minute each. The sections were then soaked in absolute methanol containing 2% H_2O_2 (v/v) for 30 minutes. At this time enzymatic digestions of some of the tissue section were performed. For the methodology, refer to Section 2.4.4. Enzymatic Degradation of Proteoglycans. Following, the sections were washed three times for 5 minutes each in TBS (0.15 M NaCl, 0.05 M Tris HCl, pH 7.6) if an IgG was used or in PBS (0.137 M NaCl, 2.7 mM KCl, 4 mM $Na_2HPO_4 \bullet 7H_2O$, 1.5 mM KH_2PO_4, pH 7.2) if an IgM was used. A wax pencil was then used to draw a circle on the glass slide around the section. Each section was then covered with 50μl of either TBS or PBS, each containing 5% normal goat serum (NGS) and incubated at room temperature for 30 minutes. The sections were then drained and covered with 50μl of either TBS/5% NGS or PBS/5% NGS containing the specified dilution of monoclonal antibody. The control sections were covered with 50μl of nonhybridoma supernatant diluted one in five of TBS/5% NGS or PBS/5% NGS. The slides were incubated in a humidified container at 6°C in the refrigerator overnight.

The next day, the sections were removed from the refrigerator and allowed 30 to 60 minutes to warm to room temperature and given four 5 minute washes with TBS or PBS respectively. Following this, the sections were incubated with 50 μl of a 1:50

[5] Hemo-D® is a xylene substitute.

dilution of goat anti-mouse IgG $F(ab')_2$ fragments in TBS/5% NGS or PBS/5% NGS for 45 minutes at room temperature. This was followed by four 5 minute washes with TBS or PBS. A 1:300 dilution of peroxidase mouse anti-peroxidase in TBS/5% NGS or PBS/5% NGS was made and 50 μl was applied to each section. After incubation at room temperature for 30 to 35 minutes, the sections were given four 5 minute washes in TBS or PBS. The slides were then immersed in TBS containing 0.05% of diaminobenzidine tetrahydrochloride and 0.01% of H_2O_2 for 4 minutes for color to develop. The enzyme reaction was stopped by rinsing under running tap water for 10 minutes. Dehydration was performed by immersion for 10 minutes each through: (1) a graded series of ethanol in water starting at 50%, 70%, 95% , and absolute, (2) methanol, and (3) Hemo-D. The sections were mounted with glass coverslips and left to dry. A Wild Leitz Diavert inverted stage light microscope was used to examine the sections.

2.3. Cell Culture

As discussed previously, the articular disc from one side was placed in fixative for histologic and immunohistochemical study, while the disc from the other side was reserved for cell culture. The latter was immediately placed in a 7.5 cm^2 petri dish (Corning®) containing approximately 4-5 ml of Dulbecco's modified Eagles medium (DMEM) (Gibco®) with 10% fetal calf serum (FCS) (Gibco®) and antibiotics. A scalpel was used to cut the disc into three equal strips which were each further divided into three to obtain nine approximately equal squares of tissue. Five of the nine blocks of tissue were transferred to another 7.5 cm^2 petri dish containing 4-5 ml of DMEM/10% FCS.

Both petri dishes were then incubated at 37°C with a mixture of 95% O_2 to 5% CO_2 at a relative humidity of 98.6%.

After three days of incubation, cells were observed to be proliferating on the plastic surfaces immediately adjacent to the tissue explants (Plate III.2.3.A.). After seven days, the cells were observed to be confluent as a monolayer[6] (Plate III.2.3.B.).

Plate III.2.3.A.
Tissue Explant

[6] Mesenchymal cells, such as fibroblasts, are known to be *anchorage dependent*, meaning that attachment to (and usually some degree of spreading on) the substrate is a prerequisite for cellular proliferation (Freshney,1987).

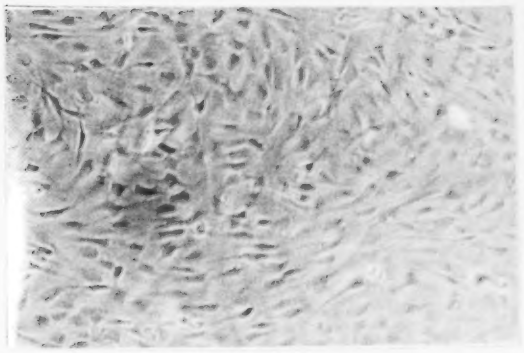


Plate III.2.3.B.
Confluent Monolayer of Cells

2.3.1. Passage of Cells

Once the cells were confluent, they were ready for passage. Following three washes with sterile PBS, to remove any serum inhibitors, the cells were treated with 0.25% trypsin[7] in PBS for 5 minutes or until the solution became cloudy indicating cell detachment. A manual pipet was used to transfer the cellular solution to a 50 ml plastic centrifuge tube (Corning) containing 10 ml of DMEM/10% FCS. The petri dishes were washed three times with 1 ml of PBS to recover any remaining cells. The wash solution was added to the first group of cells. After centrifugation for 7.5 minutes at 1500 rpm, the top liquid layer was suctioned off, leaving a small pellet of cells at the bottom of the centrifuge tube. The cells were resuspended in 10 ml of DMEM/10% FCS and centrifuged a second time in the same manner. The liquid layer was suctioned off once

[7] Trypsin is a digestive enzyme utilized to dissociate adherent cells for the purposes of counting or transfer. Furthermore, each time that the cells are exposed to trypsin, constitutes a *passage*, designated as Pη.

again. This step was necessary to allow serum inhibitors to inactivate any remaining trypsin. The cells were resuspended in 2 ml of DMEM/10% FCS and counted on a hemocytometer. The volume was adjusted to allow approximately 2 x 10^5 cells to be seeded in each 75 cm^2 flask. Twelve milliliters of DMEM/10% FCS were added to each flask.

Following incubation for five days, the medium was exchanged for fresh medium. In ten days, the cells appeared to reach confluency and were passaged once again (P2) to 150 cm^2 tissue culture flasks. In general, fresh medium (DMEM/10% FCS) was exchanged every 4 to 5 days and cells were passaged only when they were confluent.

2.3.2. Freezing and Storage of Cells

Since cell growth is exponential, it was necessary to freeze some cells for storage and later use. This procedure was performed identically to the passage technique described above with the exception of the addition of 5% dimethylsulfoxide[8] to the medium prior to freezing. A concentration of 3 x 10^6 cells/ml of DMEM/10% FCS was created to facilitate placement of 3 x 10^6 cells (1 ml) in each storage vial. The vials were then placed in a -50°C freezer for 24 hours. On the following day, the vials were immersed in liquid nitrogen for storage.

---

[8] Dimethylsulfoxide (DMSO) is a cryopreservative. In the absence of a cryopreservative, freezing of mammalian cells is lethal (Kruse and Patterson, 1973). DMSO lowers the freezing point and allows for slower cooling to minimize the damaging effects of mechanical injury by ice crystals, concentration of electrolytes, dehydration, pH changes, and denaturization of proteins (Freshney, 1987; Kruse and Patterson, 1973).

2.3.3. Collection of Conditioned Medium

Medium was collected only when the cell layer was confluent. Conditioned DMEM/10% FCS was suctioned off the layer of cells and replaced with unconditioned DMEM/2% FCS. Following three days of incubation, the conditioned medium was collected and frozen for later purification and analysis. In all cases, after DMEM/2% FCS was collected, the cells were placed on DMEM/10% FCS for a minimum of 7 days before another three day cycle of DMEM/2% FCS was put through. In this study only conditioned DMEM/2% FCS was analyzed.

2.4. Isolation and Preparation of PGs and GAGs

Proteoglycans and glycosaminoglycans from conditioned cell culture medium were analyzed. A series of extractive and purification steps were necessary to isolate the GAGs, while further enzymatic and degradative methods were necessary to prepare the GAGs for analysis.

2.4.1. DEAE Sephacel Column

The conditioned DMEM/2% FCS was passed at a rate of 3.9 ml/hr through a 1.5 x 9 cm DEAE-Sephacel column which had previously been equilibrated with column buffer [7 M urea, 0.15 M NaCl, 0.05 M Tris-HCl, 0.02% (w/v) NaN_3, and proteinase inhibitors (5 mM benzamidine hydrochloride, 1 mM PMSF, 10 mM EDTA, 0.5 mM NEM, 5 μg/l pepstatin and 5 μg/l leupeptin), pH 6.6]. The column was eluted at a flow rate of 9 ml/hr with a linear gradient of NaCl (0.15 - 1.15 M) in the same buffer.

Following, 2 M NaCl was applied to the column to release any remaining GAGs. The effluent was collected in 1 ml fractions.

2.4.2. DMB Assay

A modified Dimethylene Blue assay as described by Farndale *et al.* (1986) was performed on the DEAE column fractions. An Immulon® 1 flat bottom polystyrene plate was used to add 150 μl of DMB to 50 μl of each fraction. The plate was then read on a Multiskan spectrophotometer with a 595 nm interference filter. This procedure was used primarily to verify the presence of GAGs and also to determine when they were released from the column.

2.4.3. Dialysis and Lyophilization

Following the DMB assay, the fractions from each DEAE-Sephacel column were pooled and placed in dialysis tubing (10 000 MW pore cut off size), which had previously been boiled in distilled water for 10 min at 100°C. The samples were then placed in the cold room at 6°C and dialysed against double distilled water for seven days with changes of water 1 -2 times per day. The GAG solution was then frozen (-22°C) and lyophilized.

2.4.4. Enzymatic Degradation of Proteoglycans

2.4.4.1. Chondoitinase ABC

A GAG sample containing approximately 10 μg of uronic acid was digested with

0.05 U of chondroitinase ABC[9] from *proteus vulgaris* in 0.005 ml of buffer (0.1 M Tris-HCl, 0.1 M sodium acetate, pH 8.0, 0.02% NaN_3, pH 8.0) (Saito *et al.*, 1968). The digestion mixture was placed in a 37°C water bath for 2 hours.

This digestion produced protein cores with only the adjacent ends of the GAG chains remaining.

2.4.4.2. Chondroitinase ACII

Tissue sections were treated with chondroitinase ACII[8] prior to immunohistochemical staining. To each section, 0.01 U of enzyme in 100 μl of buffer (0.1 M Tris-HCl, 0.1 M sodium acetate, pH 7.7) was added and incubated at 37°C for 1 to 1½ hours. Three 5 minute washes followed to remove the enzyme from the sections.

This digestion was used to remove C_4S and C_6S from the sections.

2.4.4.3. Chondroitinase B

Chondroitinase B (0.001 U) was added to approximately 10 μg (10 μl) of GAG sample in 3 μl of buffer (0.1 M Tris-HCl, 0.1 M sodium acetate, 0.02% NaN_3, pH 8.0) and incubated overnight at 37°C.

This enzyme was used to remove DS from the GAG sample prior to cellulose acetate electrophoresis.

---

[9] According to the manufacturer, this enzyme exhibits degradative activity against HA as well.

2.4.4.4. Streptomyces Hyaluronidase

Streptomyces hyaluronidase enzyme was used for the immunohistochemical methods as well as for the cellulose acetate electrophoresis. Tissue sections were digested with 12.5 U of enzyme in 100 μl of buffer (0.1 M citric acid, 0.2 M disodium phosphate, pH 5.6) for 1 to 1½ hours at 37°C. The sections were then given three 5 minute washes.

To remove HA from the GAG sample prior to electrophoresis, 2.5 U of enzyme was added to 4 μg (4 μl) GAG sample in 5 μl of buffer (0.1 M Tris-HCl, 0.1 M sodium acetate, 0.02% NaN_3, pH 8.0) and incubated overnight at 37°C. The enzyme digest mixture was applied directly to the cellulose acetate strips.

2.4.4.5. Papain

The GAG sample containing approximately 80 μg (2.5 ml) of uronic acid was added to 1.1 U of papain in 2.5 ml of [2X] PBS buffer and placed in a water bath at 65°C for 4 hrs. Following, 10% trichloroacetic acid (TCA) was added and placed in a 6°C refrigerator overnight to allow proteins to precipitate.

The next day, the digestion mixture was ultracentrifuged at 13 000 rpm for 20 minutes. The supernatant was recovered and dialyzed against running tap water (cold) for 24 hours and Millipore water for another 24 hours at 6°C. Following, the sample was lyophilized and redissolved in 20 μl of water.

2.5. Enzyme Linked Immunosorbent Assay

The enzyme linked immunosorbent assay (ELISA) technique used was based on the method of Rennard *et al.* (1980) developed for analysis of other connective tissue components. Costar® flat bottom high binding EIA/RIA plates were coated with 200 ng of antigen/well (50 μl of PDS or CSPG antigen solution at a dilution of 1 μg/ml) and 50 μl of carbonate-bicarbonate coating buffer [0.05 M Na_2CO_3, 0.05 M $NaHCO_3$, 0.05% NaN_3 (w/v), pH 9.6) per well. Following incubation at room temperature for 1 to 2 hours or overnight at 4°C, the antigen and buffer solution was aspirated from each well. The wells were then incubated with 100 μl of 1% bovine serum albumin (BSA) in PBS for 1 to 2 hours at room temperature or overnight at 4°C. Following four 3 minute washes with 150 μl/well of PBST [0.137 M NaCl, 2.7 mM KCl, 4 mM $Na_2HPO_4 \bullet 7H_2O$, 1.5 mM KH_2PO_4, 0.05% NaN_3 (w/v), 0.05% Tween 20 (v/v), pH 7.2], the plates were drained and sealed for storage at -22°C. Typically, these plates were used several days to several weeks later.

After allowing the antigen coated plate 30 minutes to warm to room temperature, antigen standards (50 μl/well), PBST (50 and 100 μl/well), and the unknown sample (volume made up to 50 μl with PBST) were added to the wells. Following the addition of 50 μl of antibody solution (Table III.2.5.A), the plate was incubated overnight at a temperature of -4°C.

Ab Name	Target Ag	Ab Class	Production	Dilution	Diluent
6D6	PDS	IgG	Supernatant	1:100	PBS
CS56	CS	IgM	Ascites	1:10 000	PBS
5D4	KSPG	IgG	Ascites	1:8 000	PBS

Table III.2.5.A.
Antibodies used in the ELISAs

The next day, after the plate was warmed to room temperature and given four 3 minute washes of PBST (150 μl/well), the second antibody, an alkaline phosphate conjugated rabbit anti-mouse IgG (H&L) antiserum diluted 1:1000 in PBST (100 μl/well) was added. Following 1½ to 2 hrs of incubation at room temperature, the antibody solution was drained off and the plate was given four 3 minute washes of PBST (150 μl/well). A *p*-nitrophenylphosphate substrate in diethanolamine buffer (pH 9.8) was prepared and 100 μl was added to each well. Typically suitable color developed in 3 to 4 hrs to allow the result to be quantitated on a Multiskan equipped with a 405 nm interference filter.

2.6. Uronic Acid Analysis

Uronic acid was analyzed using a scaled down version of the method outlined by Kosakai and Yosizawa (1979) which is a modification of the carbazole method described by Bitter and Muir (1962). In this modification, 0.2 M anhydrous sodium tetraborate is

used instead of 0.025 M sodium tetraborate decahydrate resulting in only a slight reduction of color intensity of D-glucuronolactone but a substantial increase in that of L-iduronolactone (Kosakai and Yosizawa, 1979).

The glucuronolactone standards and the unknown samples were made up to a volume of 100 μl with water in 16 x 100 mm disposable glass test tubes. The tubes were placed in an ice bath while 0.6 ml of 0.2 M anhydrous sodium tetraborate was added. After boiling for 10 minutes at 100°C in a water bath, 20 μl of 0.125% carbazole in absolute ethanol was added and boiled again for 15 minutes. Color development was read at 530 nm on a Gilford 252 spectrophotometer after it was adjusted to zero with the blank standard.

2.7. Cellulose Acetate Electrophoresis

Cellulose acetate electrophoresis was performed using two different buffer systems: 0.1 M pyridine, 1.2 M glacial acetic acid, pH 3.5 (Habuchi *et al.*, 1973) and 0.1 M potassium phosphate buffer, pH 7.0 (Hata and Nagai, 1972). The advantage of the pyridine-acetic acid buffer is the ability to separate CS and DS.

After the Sepraphore ® III cellulose polyacetate strips (2.5 cm x 15.2 cm) were soaked in the buffer for a minimum of 30 minutes, the standards and unknown samples were applied over a period of approximately one minute and were allowed to air-dry between applications. The strips were positioned on a Gelman electrophoresis tray and a DC current of 1.25 AMPs was applied for 1 hour. Following this, the strips were stained in 0.1% (w/v) Alcian Blue 8GX in 0.1% (v/v) acetic acid for 3 minutes,

thoroughly washed with copious amounts of water, cleared with Sepra Clear ® II and scanned at 600 nm on a Gilford 252 spectrophotometer equipped with a linear transporter.

2.8. Di Ferrante Method for Iduronic Acid

The Di Ferrante method (Di Ferrante *et al.*, 1971) is an adaptation of the periodate-Schiff reaction.

The standards and unknown samples were made up to a volume of 250 μl with water in disposable glass 16 x 100 mm test tubes. After the addition of 50 μl of 0.5 M H_2SO_4 and 100 μl of 0.1 M $NaIO_4$, 0.2 M $NaClO_4$ in water, the mixture was incubated at 37°C in a water bath under a cool white fluorescent preparation light (40 Watt) for 1 hour. Upon removal from the water bath, 150 μl of 0.16 M Na-metabisulfate and 250 μl of Schiff's reagent (prepared fresh using the "Cold Schiff" procedure[10]) was added. After thorough mixing, the mixture was again placed in the 37°C water bath under the preparation light for 30 minutes. Optical density was read at 540 nm on a Gilford 252 spectrophotometer after it was adjusted to zero with the blank control.

---

[10] Basic Fuchsin (0.5 g) and Na-metabisulfate (0.95 g) were mixed in 25 ml of 0.25 M HCl and stirred in a aluminum foil covered beaker for 2 hours. Activated decolorizing carbon (0.25 g) was added and stirred for 2 minutes. After the solution was transferred to two test tubes, it was bench centrifuged for 8 minutes. The supernatant was transferred to another test tube containing 0.125 g activated decolorizing carbon, mixed and centrifuged again for 8 minutes. After filtration, if the supernatant was not "water" clear, the previous step was repeated another one or two times until the solution was clear.

2.9. Thin Layer Chromatography

The thin layer chromatography (TLC) technique used was that of Shimada *et al.*, 1987. Aqueous standards (Δ Di-0S, -4S, -6S) and the chondroitinase ABC digestion mixtures were applied to a 20 x 20 cm silica plate (Merck & Co.) with a micropipette. Small amounts (2 μl) were applied and dried with a hair dryer before the next application. After saturation of a chamber containing 100 ml of solvent [*n*-propanol, isopropanol, *n*-butanol, water (55:20:5:20, v/v), 0.04 M NaCl and 0.01 M ammonia] for 1 hour, the plate was placed in the chamber and left overnight to draw solvent to the top.

On the following day, the plate was air dried in a warm room (37°C) for 1 to 2 hours, sprayed with 10% H_2SO_4 in ethanol containing 0.125% carbazole, and heated in an oven for 3 minutes at 100°C. Spraying and heating were usually repeated to achieve optimal color.

The plates were immediately photographed and scanned with a Hewlett Packard ® Scanjet for computer analysis. The image of the TLC plate was scanned on a 16 shade grey scale and exported as a TIF file.

2.10. Polyacrylamide Gel Electrophoresis

The proteoglycan samples and standards were dissolved in 40 mM Tris-borate, 2% SDS (w/v), 6M urea, 10% mercapto-ethanol (v/v) and boiled 10 minutes prior to application. Aliquots (30 - 40 μl) were applied to the appropriate lanes of an SDS polyacrylamide slab gel and electrophoresed in a 0.1% SDS (w/v) Tris-borate buffer, pH 8.6 (Mechanic, 1979) for 1 hour at 50 volts, 36 mA and then at 100 volts, 74 mA for

the remainder of the run. Two gels were run at the same time on the Biorad ® Mini System; one for staining with Stains-All (Dahlberg, 1969) and the other for staining with Coomassie Blue (Mechanic, 1979).

2.11. Immunoblotting

The procedure followed for immunoblotting was identical to that of the above except smaller samples were used and upon termination of the electrophoresis, the Bromophenol Blue dye front was marked with Pyronin Y. Electrophoretic transfer to nitrocellulose was performed overnight in Tris-borate (same buffer as above without SDS) at 6 volts. The nitrocellulose paper was then incubated in 2% BSA in TBS (6D6) or PBS (CS56) for 45 minutes at 40°C. Following four 5 minute washes with TBS/PBS respectively, the sheets were incubated at room temperature for 2 hours with the primary antibodies in a 12 ml solution (6D6 1:1000, CS56 1:10 000) of TBS/PBS with 1% Tween. After four 5 minute washes with TBS/PBS, the sheets were incubated with 12 ml of a 1:1000 solution of peroxidase conjugated rabbit anti-mouse IgG antibody in TBS/PBS for 90 minutes. Again, four 5 minute washes were given and color was developed by immersing the sheets in 0.05% diaminobenzidine tetra-hydrochloride in TBS containing 0.01% H_2O_2 (w/v) and 0.033% cobalt chloride (w/v) for 10 to 12 minutes. The sheets were washed several times with large amounts of water and air dried between sheets of filter paper.

CHAPTER FOUR

RESULTS

RESULTS

OUTLINE

1. Histology and Immunohistochemistry 112

1.1. Histology 112

1.1.1. Haematoxylin and Eosin 112

1.1.2. Masson's Trichrome 116

1.1.3. Taenzer-Unna Orcein 118

1.1.4. Alcian Blue and Safranin-*O* 120

1.1.5. Alcian Blue and Enzymatic Degradation 123

1.2. Immunohistochemistry 125

1.2.1. Chondroitin Sulphate 126

1.2.2. Dermatan Sulphate Proteoglycan II 128

1.2.3. Keratan Sulphate 130

1.2.4. Hyaluronic Acid 132

2. Enzyme Linked Immunosorbent Assays 134

2.1. Chondroitin Sulphate Proteoglycan 135

2.2. Dermatan Sulphate Proteoglycan II 144

2.3. Keratan Sulphate Proteoglycan 149

3. Uronic Acid Analysis 151

3.1. Uronic Acid Content 151

4. Iduronic Acid Analysis 152

4.1. Iduronic Acid Content 152

5. Cellulose Acetate Electrophoresis 154

5.1. GAG Representation 154

6. Thin Layer Chromatography 162

6.1. Chondroitin Sulphate Representation 162

7. Gel Electrophoresis and Immunoblotting 164

7.1. Polyacrylamide Gel Electrophoresis 164

7.2. Immunoblotting 166

1. Histology and Immunohistochemistry

1.1. Histology

A variety of stains were utilized to study the morphology and composition of the articular disc. Histologic examination of the articular disc confirmed a definite biconcave structure composed of dense avascular fibrous tissue enriched with large amounts of GAGs.

1.1.1. Haematoxylin and Eosin

Haematoxylin and Eosin were used with the Haematoxylin component staining the cell nuclei blue-black, while the Eosin stained cell cytoplasm and most connective tissues in varying shades and intensities of pink, orange and red (Plate IV.1.1.1.A.).

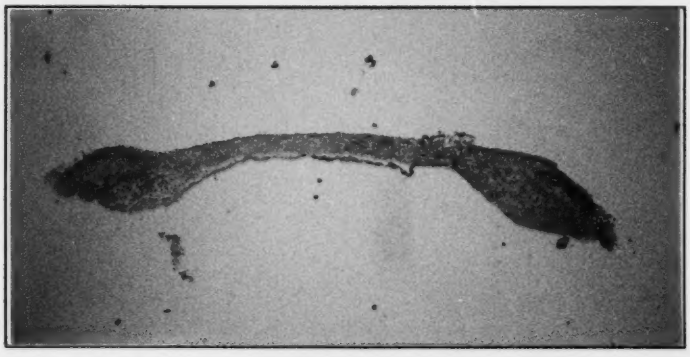


Plate IV.1.1.1.A.
TMJ Articular Disc - Haematoxylin and Eosin
(4X Magnification)

In general, the disc was highly cellular, however, the concentration of cells appeared to be denser in the intermediate band compared to the anterior and posterior bands (Plate IV.1.1.1.B.). A count of cells in the anterior, intermediate and posterior bands produced a ratio of 1:1.5:1, respectively. Along the inferior surface of the intermediate band were a distinct layer of cells as well as capillary-like structures which contained red blood cells. An immunohistochemical stain for Factor VIII-related antigen[1] was performed (Department of Pathology at the University of Alberta Hospital, Edmonton, Alberta) to confirm the presence of capillaries (Plate IV.1.1.1.C.). The positive and negative controls for this stain was adult human ovary and human hemangioma, respectively. Unfortunately, the results of this method were not definitive for capillaries. Lastly, a zone of relative acellularity was evident between the inferior surface of the intermediate band of the disc and the rest of the disc.

[1] Factor VIII-related antigen (VIII Rag) is a glycoprotein synthesized by endothelial cells and megakaryocytes (Nadji *et al.*, 1980; Jaffe, 1977; Nachman *et al.*, 1977).

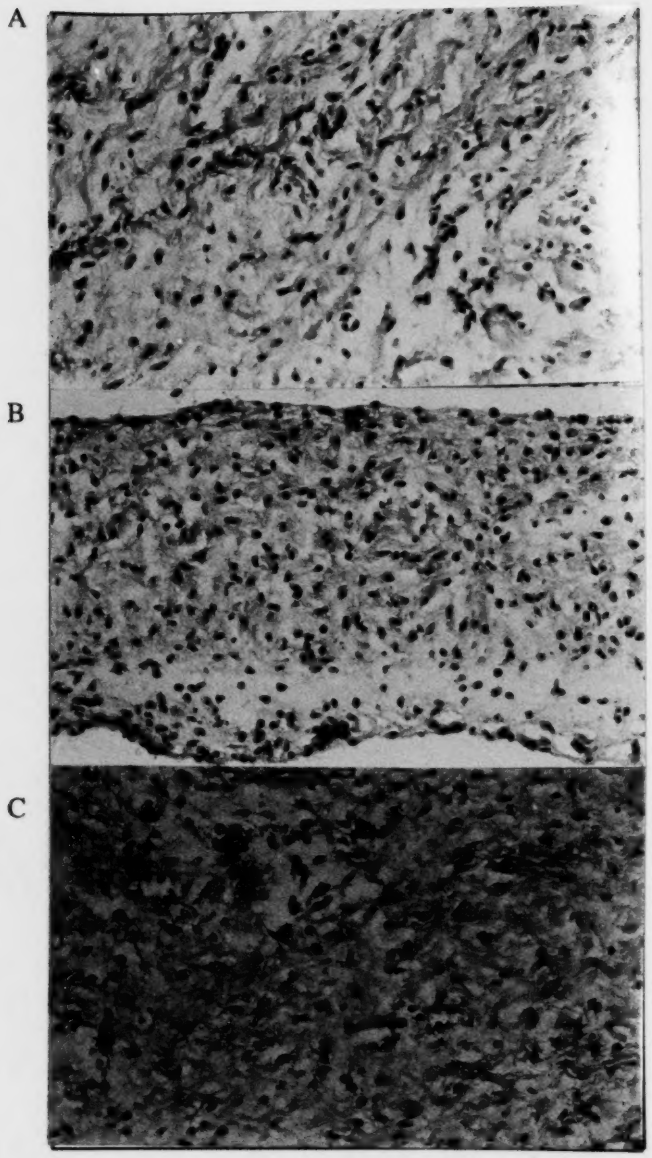


Plate IV.1.1.1.B.

TMJ Articular Disc - Haematoxylin and Eosin (100X Magnification)
A = Anterior Band, B = Intermediate Band, C = Posterior Band

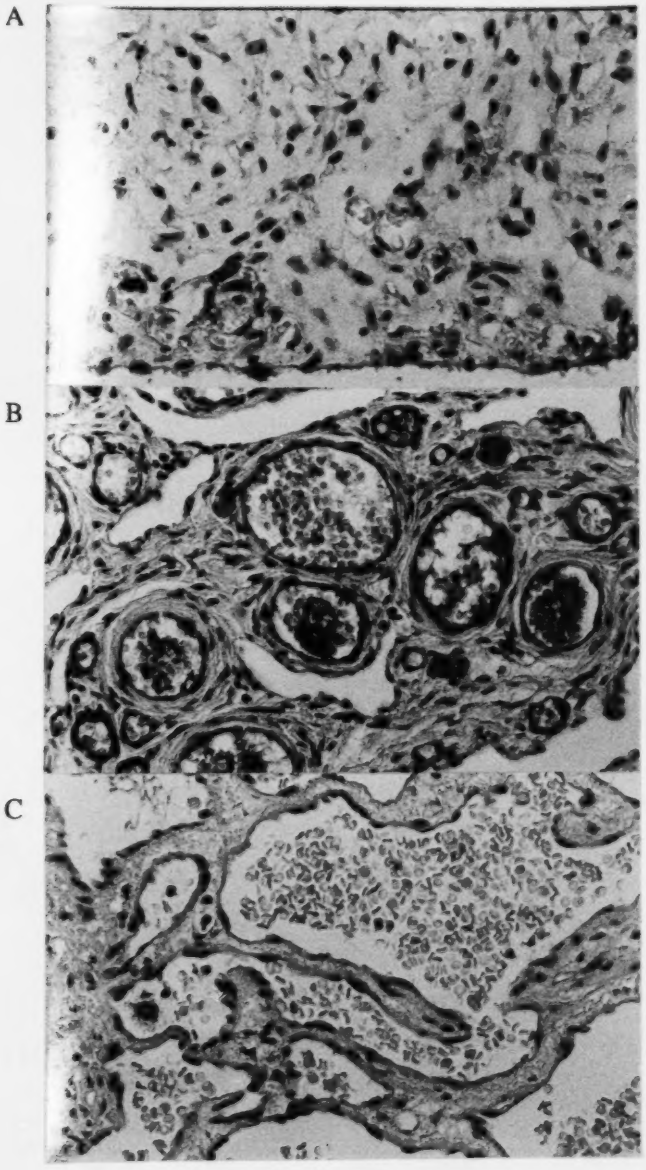


Plate IV.1.1.1.C.

Immunohistochemical Stain for Factor VIII-Related Antigen
A = Intermediate Band of TMJ Articular Disc (160X), B = Positive Control - Human Ovary (100X), C = Negative Control - Human Hemangioma (100X)

1.1.2. Masson's Trichrome

Masson's Trichrome is a stain which gives collagen fibers a greenish-blue hue while it stains cytoplasm, nucleii and some intercellular fibers red. This stain revealed an abundance of collagen fibres distributed throughout the disc. (Plate IV.1.1.2.A.).

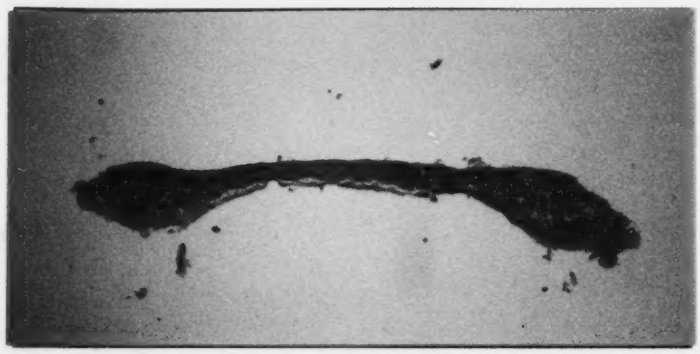


Plate IV.1.1.2.A.
TMJ Articular Disc - Masson's Trichrome (4X Magnification)

Bands of collagen fibers were seen arranged in parallel arrays (Plate IV.1.1.2.B.). The direction of these bands appeared to be in an anteroposterior direction, however, one must keep in mind that these are sagittal sections and collagen fibers oriented in a plane other than sagittal would appear somewhat randomly arranged.

The distribution of collagen fibers was not uniform. There was a marked increase in density of collagen fibers in the intermediate band of the articular disc (Plate IV.1.1.2.B).

As with the Haematoxylin and Eosin stain, there was a denser arrangement of cells in the intermediate band compared to the anterior and posterior bands. As well, a

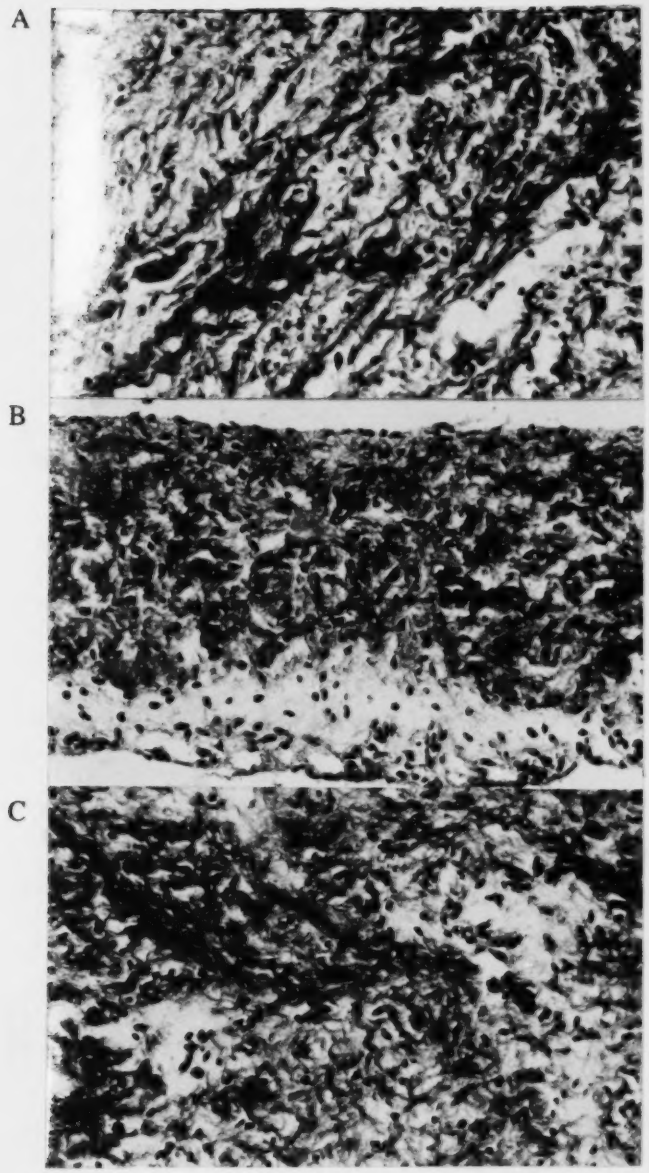


Plate IV.1.1.2.B.
TMJ Articular Disc - Masson's Trichrome (100X Magnification)
A = Anterior Band, B = Intermediate Band, C = Posterior Band

distinct layer of cells and capillaries were visualized on the inferior surface of the intermediate band.

1.1.3. Taenzer-Unna Orcein

Taenzer-Unna Orcein stain was used to detect elastic fibers as it stains them a brownish-black. Elastic fibers were not as apparent as collagen fibers. They were not visible under low magnification (Plate IV.1.1.3.A.), however, under higher magnification, they were observed (Plate IV.1.1.3.B). Elastic fibers were detected in the anterior and posterior bands of the articular disc. Relative to the collagen fibers, the elastic fibers appeared to be very small. Furthermore, they did not appear to have any type of regular arrangement.

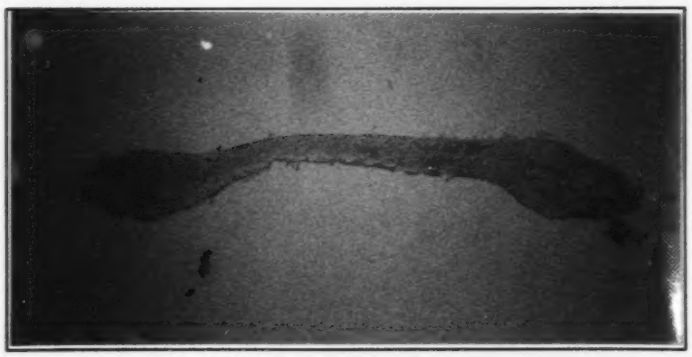


Plate IV.1.1.3.A.
TMJ Articular Disc - Taenzer-Unna Orcein (4X Magnification)

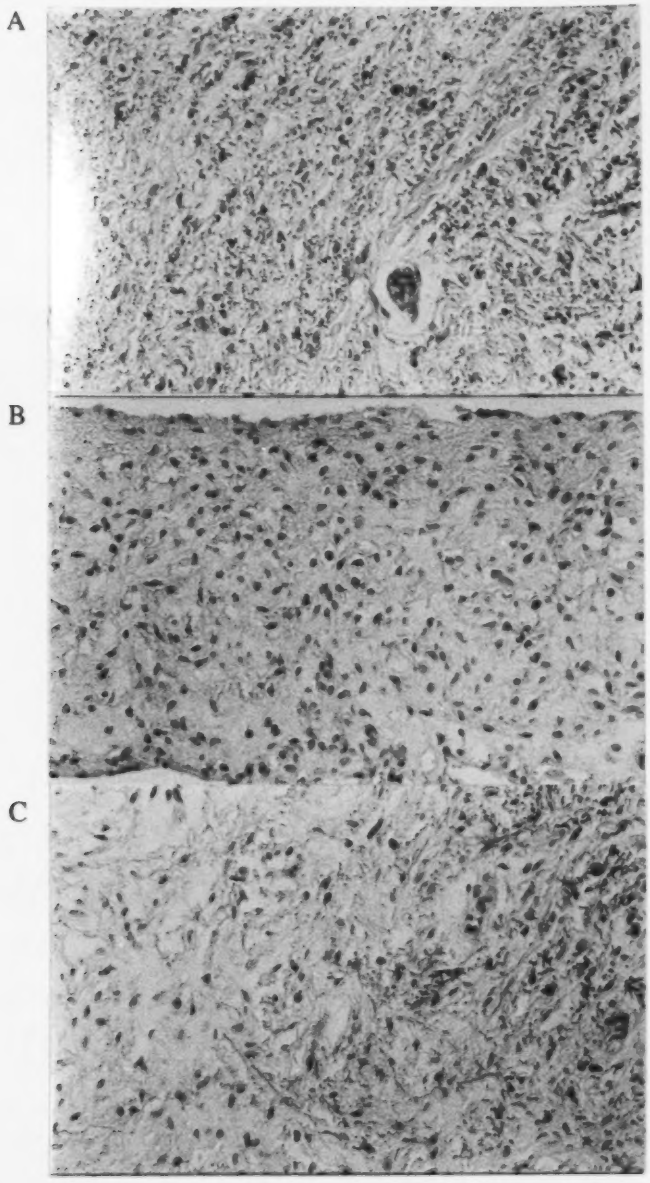


Plate IV.1.1.3.B.

TMJ Articular Disc -Taenzer-Unna Orcein (100X Magnification)
A = Anterior Band, B = Intermediate Band, C = Posterior Band

1.1.4. Alcian Blue and Safranin-*O*

Alcian Blue and Safranin-*O* stains were used to illustrate the rich content of GAGs in the articular disc. Alcian Blue stained GAGs blue (Plate IV.1.1.4.A.) while Safranin-*O* stained them pink to red (Plate IV.1.1.4.B.). Note that the staining pattern with Alcian Blue is relatively uniform, while Safranin-*O* shows more intense staining in the central area of the disc.

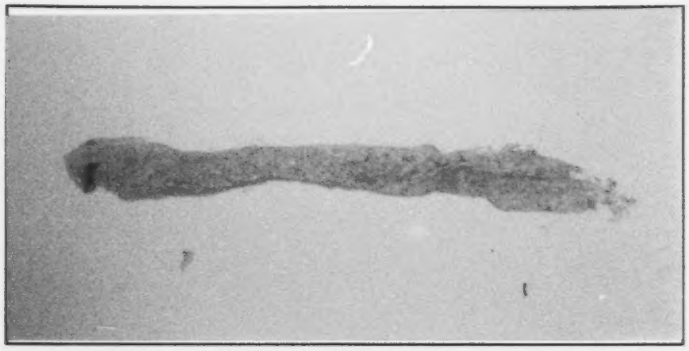


Plate IV.1.1.4.A.
TMJ Articular Disc - Alcian Blue (4X Magnification)

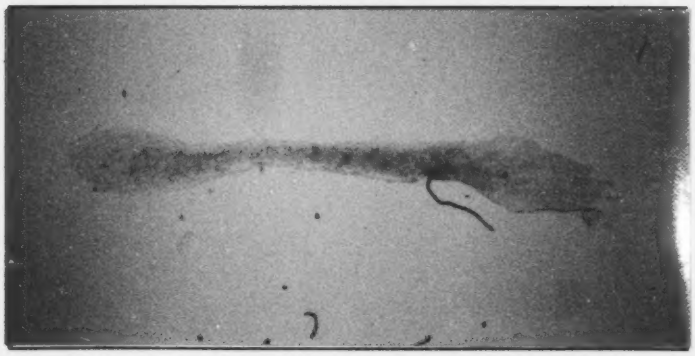


Plate IV.1.1.4.B.
TMJ Articular Disc - Safranin-*O* (4X Magnification)

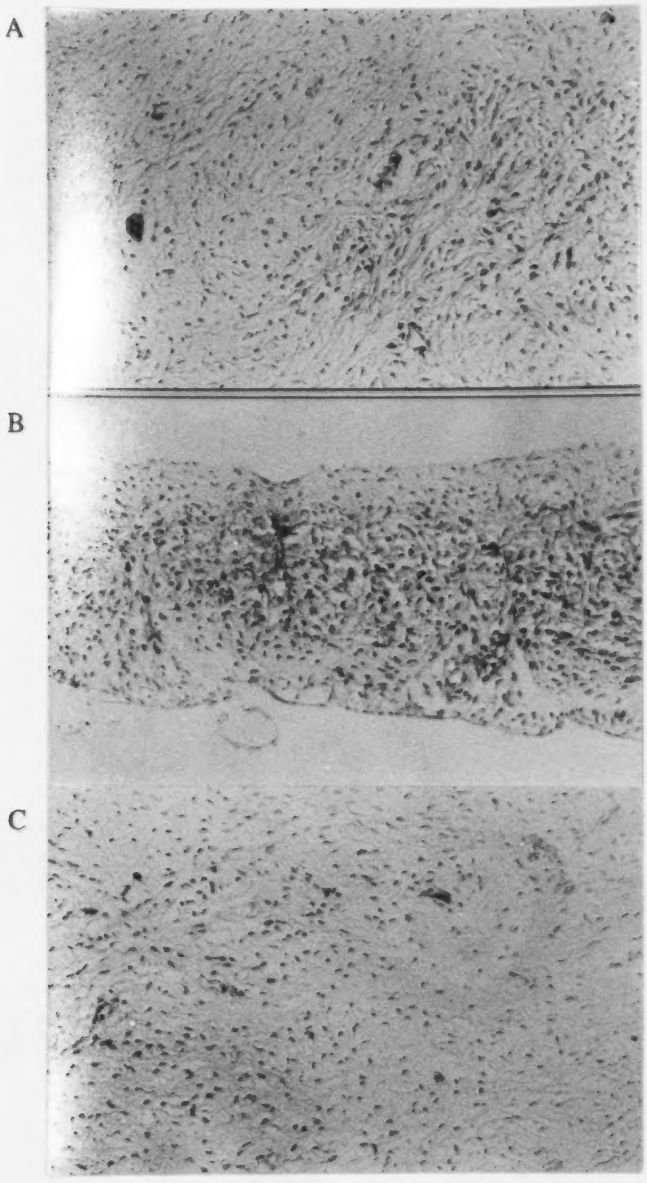


Plate IV.1.1.4.C.

TMJ Articular Disc - Safranin-*O* (64X Magnification)

A = Anterior Band, B = Intermediate Band, C = Posterior Band

Under higher magnification, Safranin-*O* staining was observed to be more intense in the deeper zones of the disc and less intense in the superficial layers (Plate IV.1.1.4.C.). This intense staining seems to correspond with the areas of dense cellularity observed with the Haematoxylin and Eosin and Masson's Trichrome stains.

1.1.5. Alcian Blue and Enzymatic Degradation

Following enzymatic degradation, the disc sections were stained with Alcian Blue and the peak spectral absorbance of the sections was read on a spectrophotometer at 600 nm (Plate IV.1.1.5.A). This technique seemed to be useful for sections of tissue that were relatively homogenous such as the articular disc, however tissues such as skin and condylar cartilage with separations and layers produced inconsistent and artifactual readings which are designated with a hyphen in Table IV.1.1.4.A..

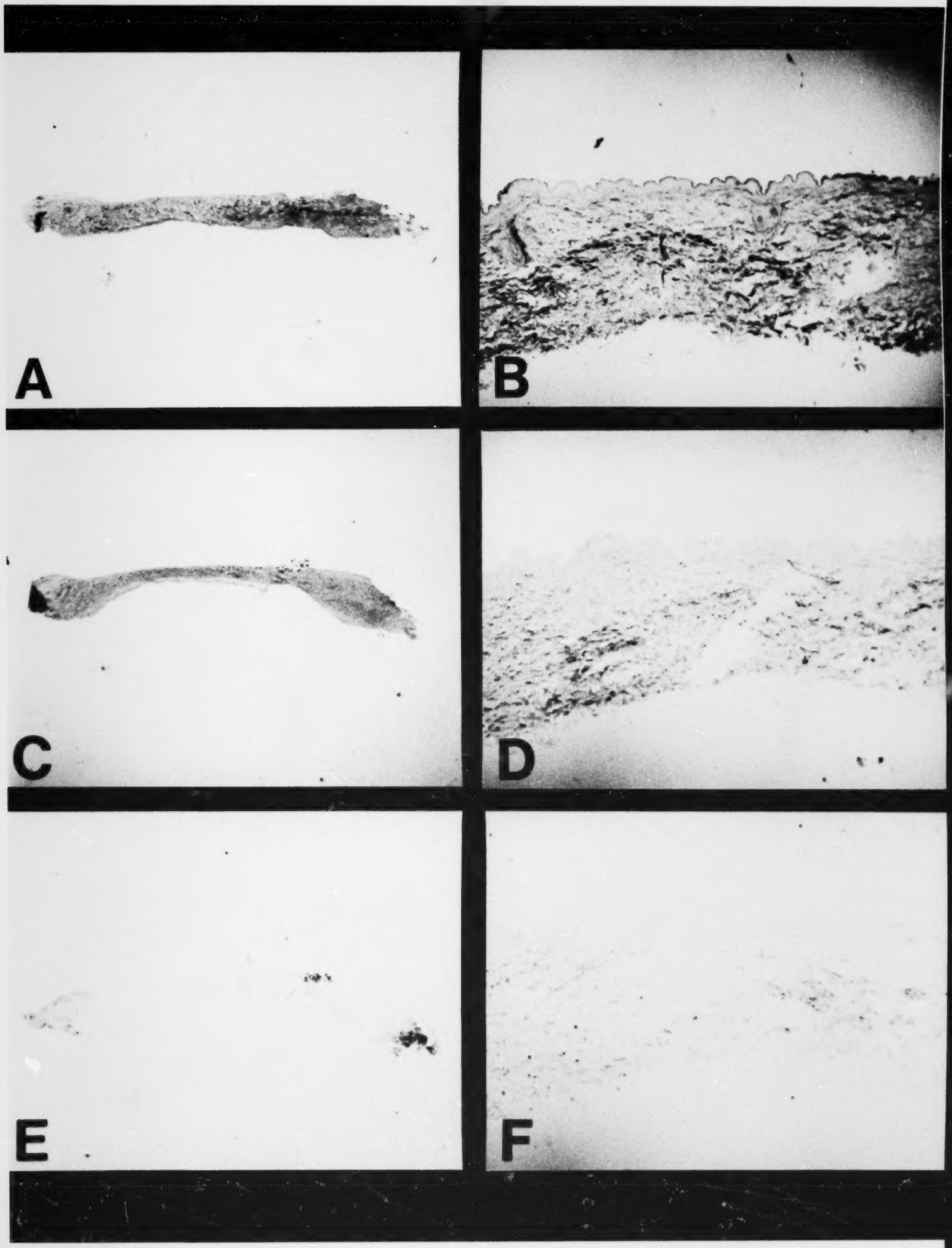
Tissue	Enzyme	Stain	Absorbance
disc	none	none	0.004
human skin	none	none	-
fetal cond. cart.	none	none	-
disc	none	Alcian Blue	0.448
human skin	none	Alcian Blue	-
fetal cond.cart.	none	Alcian Blue	-
disc	S.H.	Alcian Blue	0.415
human skin	S.H.	Alcian Blue	-
fetal cond.cart.	S.H.	Alcian Blue	-
disc	ACII	Alcian Blue	0.200
human skin	ACII	Alcian Blue	-
fetal cond.cart.	ACII	Alcian Blue	-

Table IV.1.1.4.A.
Spectral Absorbance of Alcian Blue Stained Sections
(S.H. = *Streptomyces* Hyaluronidase, ACII = Chondroitinase ACII)

Plate IV.1.1.5.A.

Alcian Blue Staining of Articular Disc and Human Skin

A = Articular Disc, No Enzyme, Alcian Blue Stain; B = Human Skin, No Enzyme, Alcian Blue Stain; C = Articular Disc, *Streptomyces* Hyaluronidase, Alcian Blue Stain; D = Human Skin, *Streptomyces* Hyaluronidase, Alcian Blue Stain; E = Articular Disc, Chondroitinase ACII, Alcian Blue Stain; F = Human Skin, Chondroitinase ACII, Alcian Blue Stain.


A
B
C
D
E
F

As mentioned above, this technique seemed to work well only with homogenous tissues such as the articular disc, as readings for the articular disc were the only ones that were reproducible. Therefore calculations were performed for the disc readings only.

Calculations were performed after subtracting the blank (unstained) absorbance value of the articular disc (0.004) from the reading. Digestion with *Streptomyces* hyaluronidase decreased absorbance of the articular disc by 7.4%. Since this enzyme is specific for HA, one may assume that this result corresponds to an equivalent decrease in HA. However, because HA is not sulphated, it does not stain as intensely as sulphated GAGs with alcian blue. A compensation factor for HA (0.55) was determined with the use of standards in previous work in Dr. Scott's laboratory. Therefore, the GAG content following digestion with *Streptomyces* hyaluronidase, decreased by 13.5%.

Following enzymatic treatment with chondroitinase ACII, the absorbance of the disc decreased by 55%. However, this enzyme exhibits activity for both CS and HA. Therefore if one were to subtract the 7.4% value of HA from 55%, then it would appear that a 47.6% reduction in absorbance is due to removal of CS. In terms of relative GAG proportions, it would appear that the HA:CS ratio is approximately 1:3.5.

1.2. Immunohistochemistry

In all trials, control sections of the fetal articular disc incubated with non-immune serum were used as well as control sections of other tissues. To measure the specificity of the antibodies, some sections were treated with degradative enzymes for the various GAGs prior to staining.

Staining intensity was judged qualitatively using a scale of: 0, +, ++, +++, ++++ where 0 represents no detectable staining, + = staining just detectable, ++ = mild staining, +++ = moderate staining, ++++ = intense staining.

1.2.1. Chondroitin Sulphate

Monoclonal antibody (clone CS56) was used to detect the presence of chondroitin sulphate. Staining was observed to be uniform throughout the TMJ articular disc. To verify immunological reaction to CS, the enzyme chondroitinase ACII was used on some sections to digest CS prior to staining.

Tissue	Enzyme	Antibody	Staining
fetal disc	none	non-imm. serum	0
bovine skin	none	non-imm. serum	0
bov. nasal cart.	none	non-imm. serum	0
fetal disc	none	CS56	++++
bovine skin	none	CS56	+++
bov. nasal cart.	none	CS56	+++
fetal disc	ACII	CS56	+
bovine skin	ACII	CS56	+
bov. nasal cart.	ACII	CS56	+

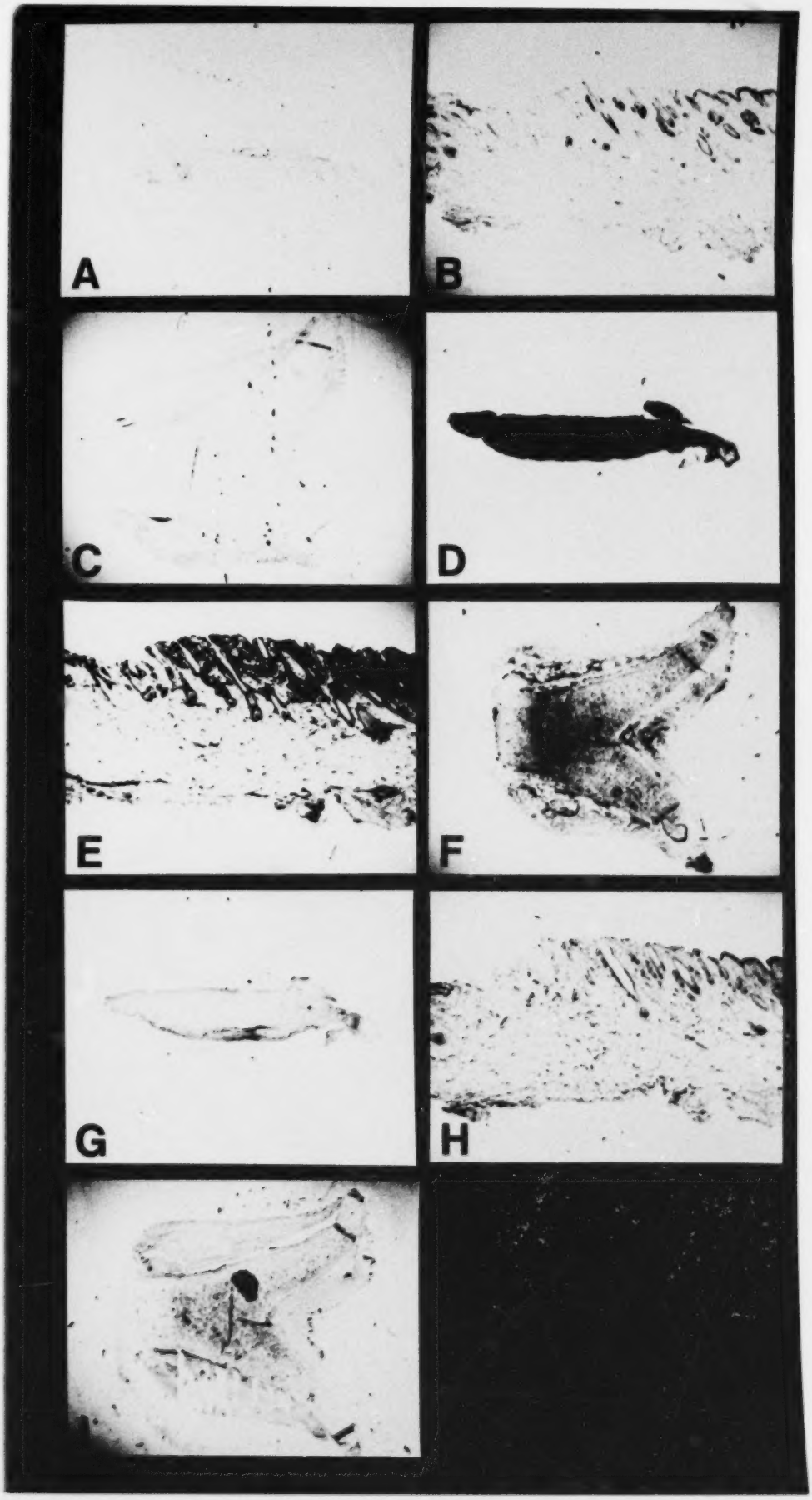
Table IV.1.2.1.A.
Chondroitin Sulphate Staining in Tissue Sections
(ACII = Chondroitinase ACII, CS56 = Monoclonal Antibody CS56)

Staining intensity was significantly reduced although not eliminated with enzymatic treatment.

Plate IV.1.2.1.A.

Chondroitin Sulphate Staining in Tissue Sections

A = Fetal Disc, No Enzyme, No Antibody; B = Bovine Skin, No Enzyme, No Antibody; C = Bovine Nasal Cartilage, No Enzyme, No Antibody; D = Fetal Disc, No Enzyme, CS56; E = Bovine Skin, No Enzyme, CS56; F = Bovine Nasal Cartilage, No Enzyme, CS56; G = Fetal Disc, Chondroitinase ACII, CS56; H = Bovine Skin, Chondroitinase ACII, CS56; I = Bovine Nasal Cartilage, Chondroitinase ACII, CS56.


A
B
C
D
E
F
G
H

1.2.2. Dermatan Sulphate Proteoglycan II

The monoclonal antibody 6D6 was used to detect dermatan sulphate proteoglycan II (PDS). The enzyme chondroitinase ACII was used with some tissue sections to remove CS prior to staining with 6D6. The rationale was to determine if CS was interfering with the immunological activity of 6D6.

Tissue	Enzyme	Antibody	Staining
fetal disc	none	non-imm. serum	0
human skin	none	non-imm. serum	0
bovine skin	none	non-imm. serum	0
fetal disc	none	6D6	+
human skin	none	6D6	++++
bovine skin	none	6D6	++++
fetal disc	ACII	6D6	+
human skin	ACII	6D6	++++

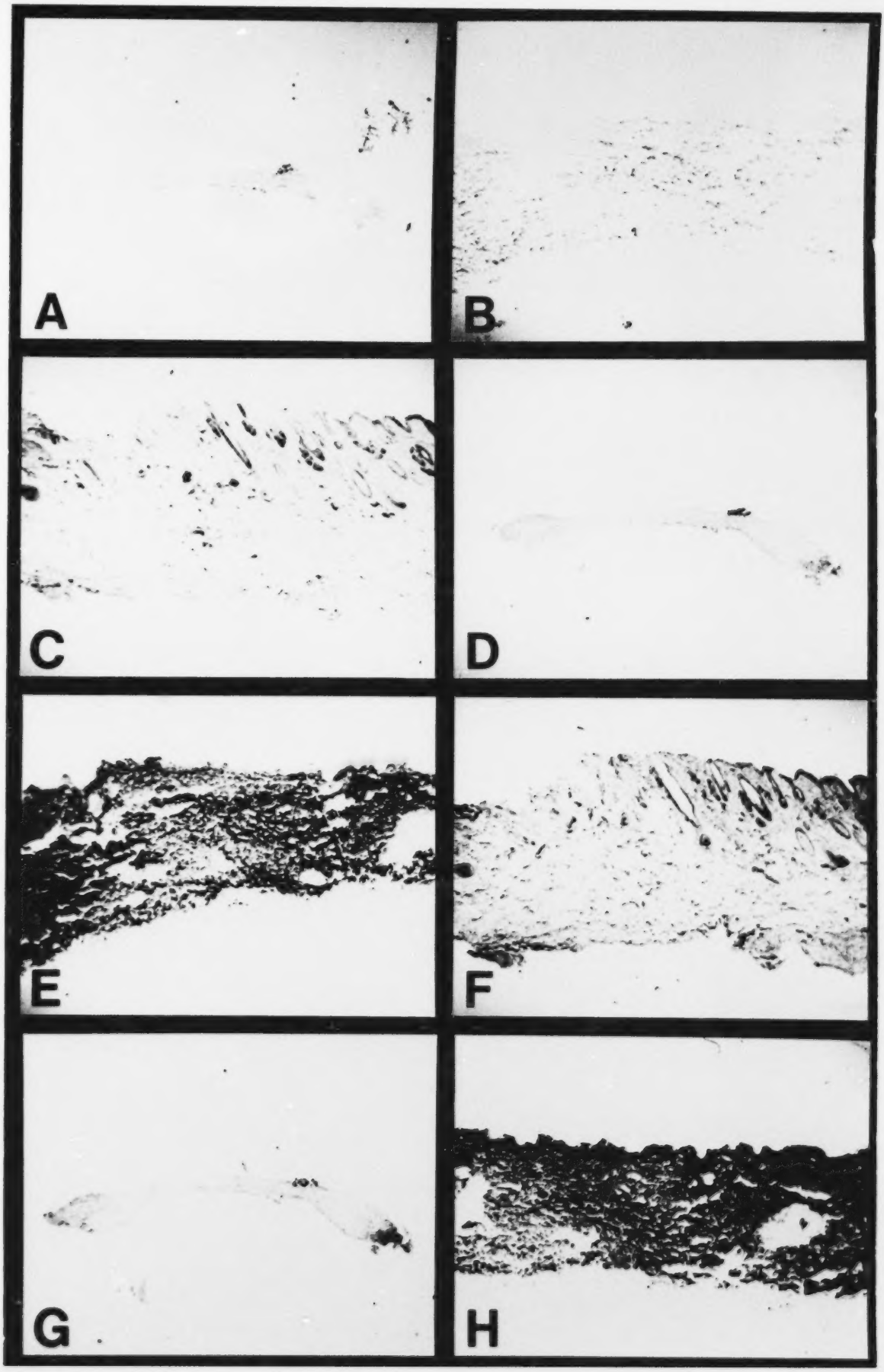
Table IV.1.2.2.A.
Dermatan Sulphate Proteoglycan II Staining in Tissue Sections
(ACII = Chondroitinase ACII, 6D6 = Monoclonal Antibody 6D6)

Light staining was seen in the articular disc in relation to the other tissues (Plate IV.1.2.2.A.). The staining was not uniform with more intense staining occurring in the edges of the intermediate band. The enzyme chondroitinase ACII did not have an effect on staining of the sections with antibody 6D6.

Plate IV.1.2.2.A.

DSPG II Staining in Tissue Sections

A = Fetal Disc, No Enzyme, No Antibody; B = Human Skin, No Enzyme, No Antibody; C = Bovine Skin, No Enzyme, No Antibody; D = Fetal Disc, No Enzyme, 6D6; E = Human Skin, No Enzyme, 6D6; F = Bovine Skin, No Enzyme, 6D6; G = Fetal Disc, Chondroitinase ACII, 6D6; H = Human Skin, Chondroitinase ACII, 6D6.


A
B
C
D
E
F
G
H

1.2.3. Keratan Sulphate

The monoclonal antibody 5D4, developed by Caterson *et al.* (1985) was used to detect keratan sulphate.

Tissue	Enzyme	Antibody	Staining
fetal disc	none	non-imm. serum	0
bovine skin	none	non-imm. serum	0
bov. nasal cart.	none	non-imm. serum	0
fetal disc	none	5D4	0
bovine skin	none	5D4	+
bov. nasal cart.	none	5D4	++

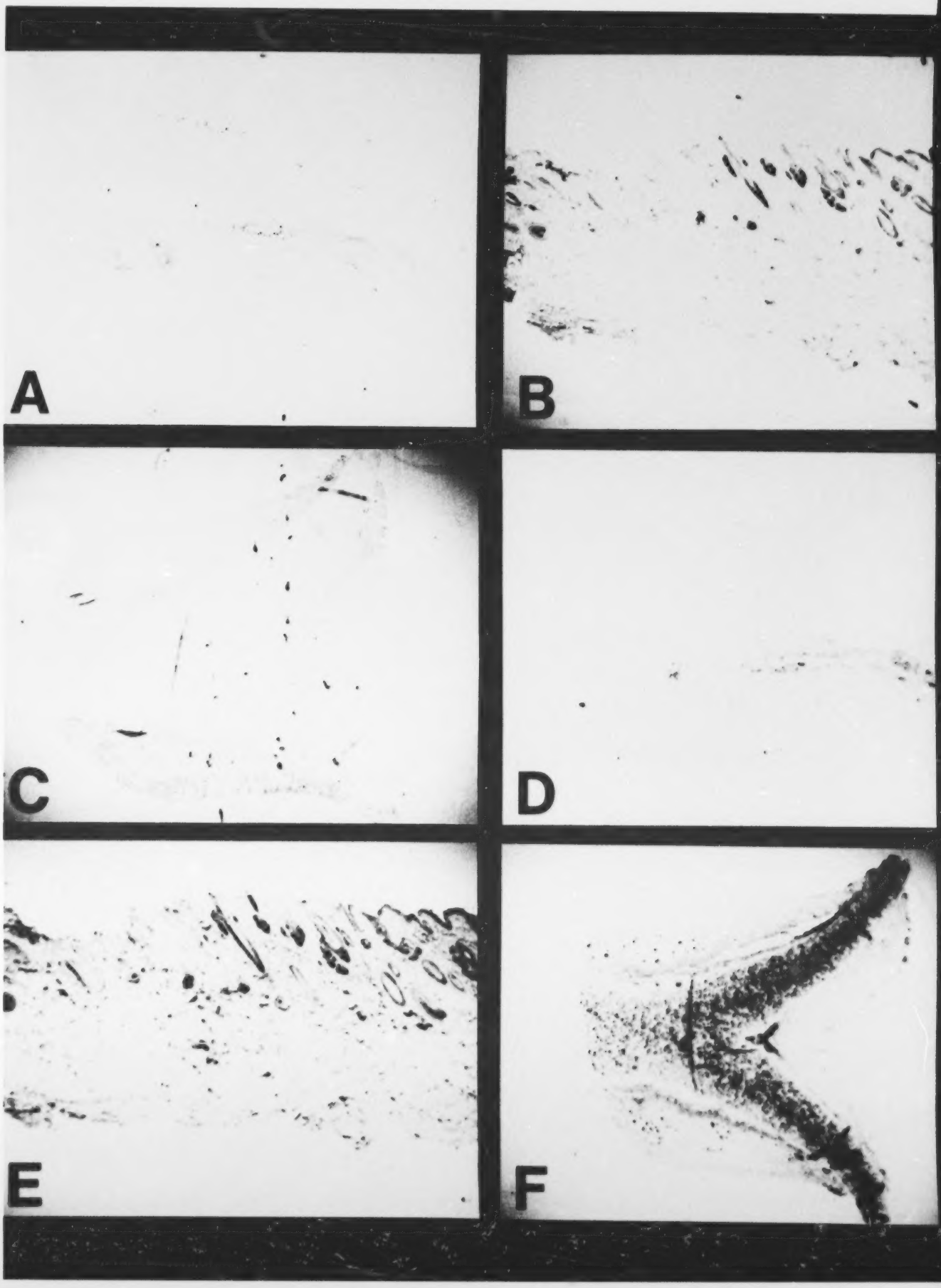
Table IV.1.2.3.A.
Keratan Sulphate Staining in Tissue Sections
(5D4 = Monoclonal Antibody 5D4)

Staining for keratan sulphate was not evident in the fetal TMJ articular disc (Plate IV.1.2.3.A.).

Plate IV.1.2.3.A.

Keratan Sulphate Staining in Tissue Sections

A = Fetal Disc, No Antibody; B = Bovine Skin, No Antibody; C = Bovine Nasal Cartilage, No Antibody; D = Fetal Disc, 5D4, E = Bovine Skin, 5D4; F Bovine Nasal Cartilage, 5D4.


A
B
C
D
E
F

1.2.4. Hyaluronic Acid

The antibody to hyaluronic acid, NDOG1 (Sigma® MCA-277) was used to observe HA in the tissues. *Streptomyces* hyaluronidase was used to degrade HA.

Tissue	Enzyme	Antibody	Staining
fetal disc	none	non-imm. serum	0
human skin	none	non-imm. serum	0
fetal disc	none	NDOG1	+++
human skin	none	NDOG1	+++
fetal disc	S.H.	NDOG1	++
human skin	S.H.	NDOG1	++
fetal disc	ACII	NDOG1	++
human skin	ACII	NDOG1	++
fetal disc	ACII & S.H.	NDOG1	+
human skin	ACII & S.H.	NDOG1	+

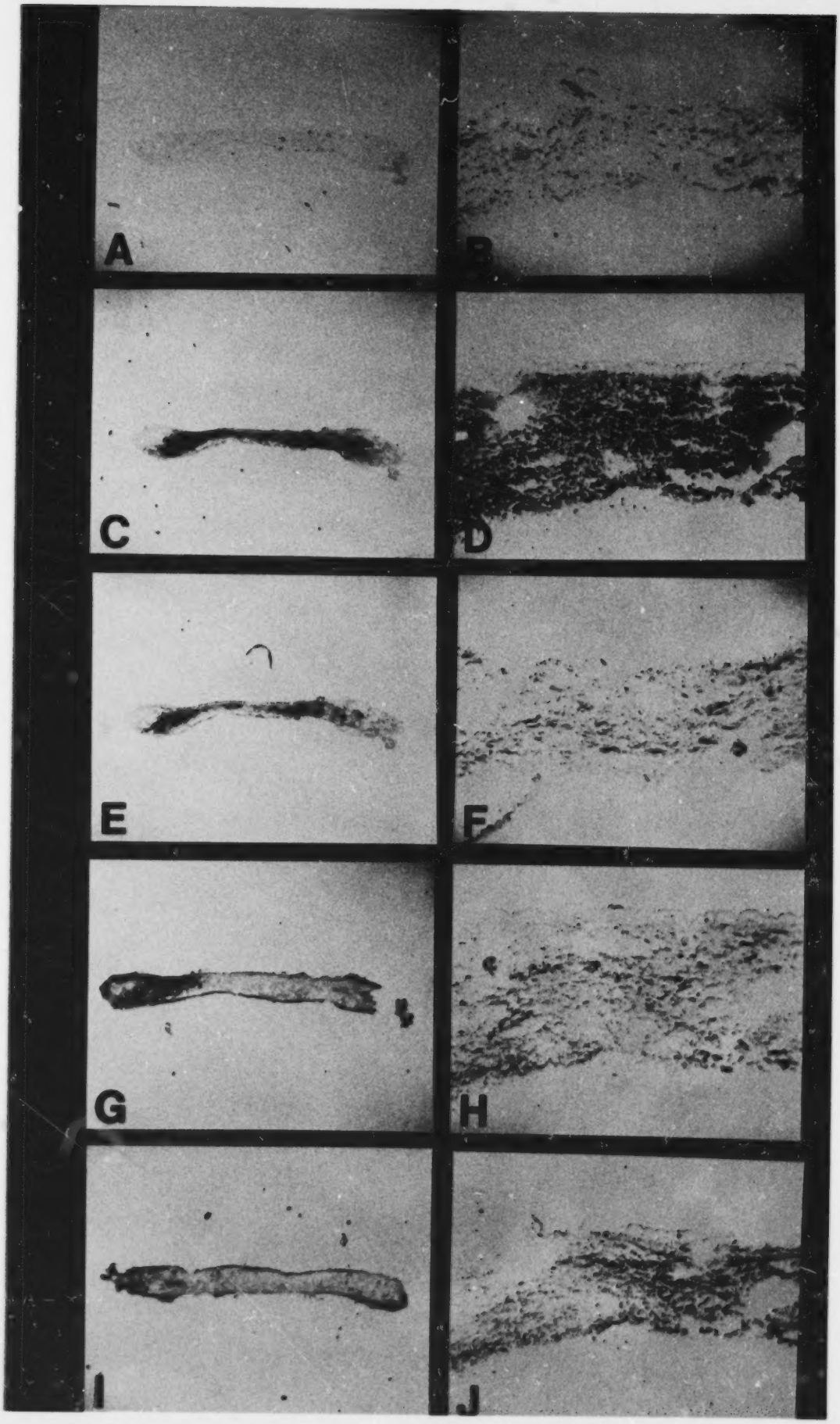
Table IV.1.2.4.A.
Hyaluronic Acid Staining in Tissue Sections
(S.H. = *Streptomyces* hyaluronidase, ACII = Chondroitinase ACII)
(NDOG1 = Monoclonal Antibody NDOG1)

Staining was observed to be uniform throughout the articular disc. Following digestion with *Streptomyces* hyaluronidase, staining was only slightly reduced, prompting us to believe that the HA antibody was not specific to HA and may be reacting with another GAG such as chondroitin sulphate. Following a digestion with the enzyme chondroitinase ACII, staining was also slightly reduced. Lastly, after a double digestion starting with chondroitinase ACII followed by *Streptomyces* hyaluronidase, staining was significantly reduced although not eliminated.

Plate IV.1.2.4.A.

Hyaluronic Acid Staining in Tissue Sections

A = Fetal Disc, No Enzyme, No Antibody; B = Human Skin, No Enzyme, No Antibody; C = Fetal Disc, No Enzyme, NDOG1; D = Human Skin, No Enzyme, NDOG1; E = Fetal Disc, *Streptomyces* Hyaluronidase, NDOG1; F = Human Skin, *Streptomyces* Hyaluronidase, NDOG1; G = Fetal Disc, Chondroitinase ACII, NDOG1; H = Human Skin, Chondroitinase ACII, NDOG1; I = Fetal Disc, Chondroitinase ACII & *Streptomyces* Hyaluronidase, NDOG1; J = Human Skin, Chondroitinase ACII & *Streptomyces* Hyaluronidase, NDOG1.


A
B
C
D
E
F
G
H
I
J

2. Enzyme Linked Immunosorbent Assays

As mentioned in the materials chapter, each collection of cell culture medium was categorized according to the order in which it was run through the DEAE-Sephacel column. Further details may be found in Table IV. 2.0.A.

DEAE Column #	Source and Passage	FCS Content (%)	Initial Volume (ml)	Reconstitute Volume (ml)
DEAE1	FD1-P3	2	220	5.5
DEAE2	FD2-P2	2	220	2.5
DEAE3	FD2-P2	0	220	2.5
DEAE4	Unconditioned	2	220	2.5
DEAE5	FD2-P2	2	555	3.0
DEAE6	FD1-P3	2	465	3.0

Table IV.2.0.A.
Description of Cell Culture Medium

ELISAs were performed primarily for CSPG and DSPG II on all the DEAE-Sephacel column collections. An ELISA was performed on DEAE5 for KS as well. Following the assays, curves were plotted and calculations were made taking into account any dilutions of the unknown sample, the original volume and the reconstituted volume to determine the amount of antigen present in the sample. For purposes of comparison, the amount of antigen is expressed per 100 ml of unconcentrated medium.

2.1. Chondroitin Sulphate Proteoglycan

The monoclonal antibody clone CS56 was used at a dilution of 1: 10 000 for all the ELISAs. It was observed that even at this dilution, CS56 exhibited strong reactivity for the DEAE column material. As a result, the unknown samples were often diluted 5, 7, 14 or 30 times prior to the assay.

Relatively large amounts of CSPG were found in DEAE1, 5 and 6 (Figure IV.2.1.A.,C.) while considerably smaller amounts were found in the other samples (Figure IV.2.1.B.). Table IV.2.1.A. compares the CSPG content of the column material (expressed per 100 ml of culture medium).

DEAE1	DEAE2	DEAE3	DEAE4	DEAE5	DEAE6
3.4 mg	538 μg	194 μg	308 μg	4.5 mg	2.5 mg

Table IV.2.1.A.
CSPG Content /100 ml Culture Medium

During the course of the experiments, questions arose relating to the specificity of clone CS56. As an initial step, an ELISA was performed to antigenic standards of C_4S, C_6S, PDS and HA (Figure IV.2.1.D.). It was discovered that binding of CS56 to CSPG from bovine TMJ articular disc was strongly inhibited by C_6S, very little by C_4S, and not at all by either PDS or HA. Although it was reassuring that CS56 was not reacting with PDS or HA, it was surprising to find that CS56 had very little activity towards C_4S. This relationship was explored further and confirmed with a subsequent

ELISA (Figure IV.2.1.E.). Furthermore, a question was raised concerning the validity of using a CSPG standard versus a CS standard. Further assays were performed to demonstrate the relationship between the two standard curves (Figure IV.2.1.F.,G.). Figure IV.2.1.F. and Figure IV.2.1.G. represent replicate trials. It was found that the two curves were fairly close to each other with the IC_{50} of the CSPG curve being slightly higher than that of the C_6S standard curve.

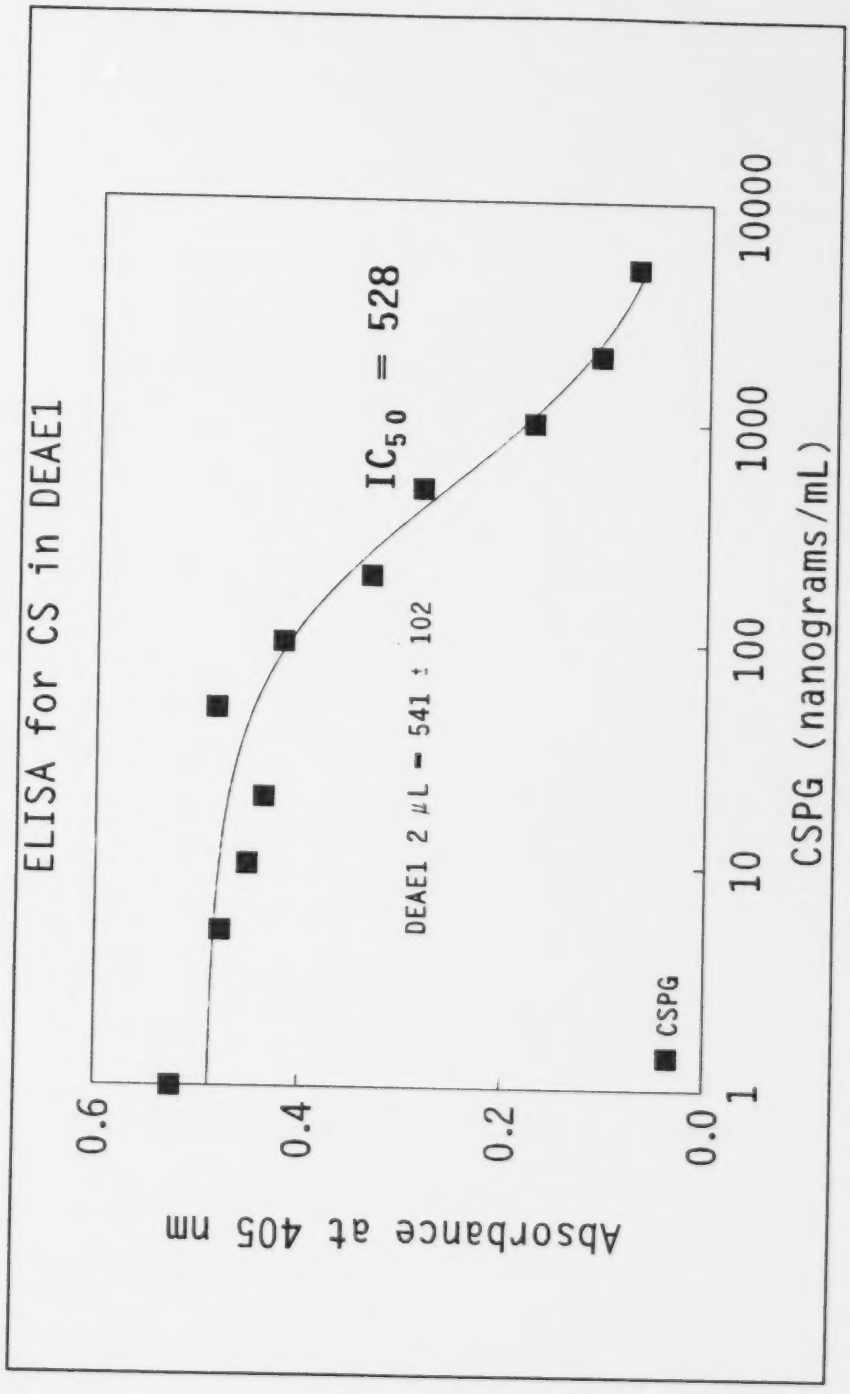


Figure IV.2.1.A.
ELISA for CS in DEAE 1
CSPG from adult bovine TMJ articular disc was used to coat the ELISA plate as well as for the antigenic standards. Antibody CS56 was used at a dilution of 1:10 000 in PBST. The DEAE1 sample was duplicated with a mean values of 541 ± 102.

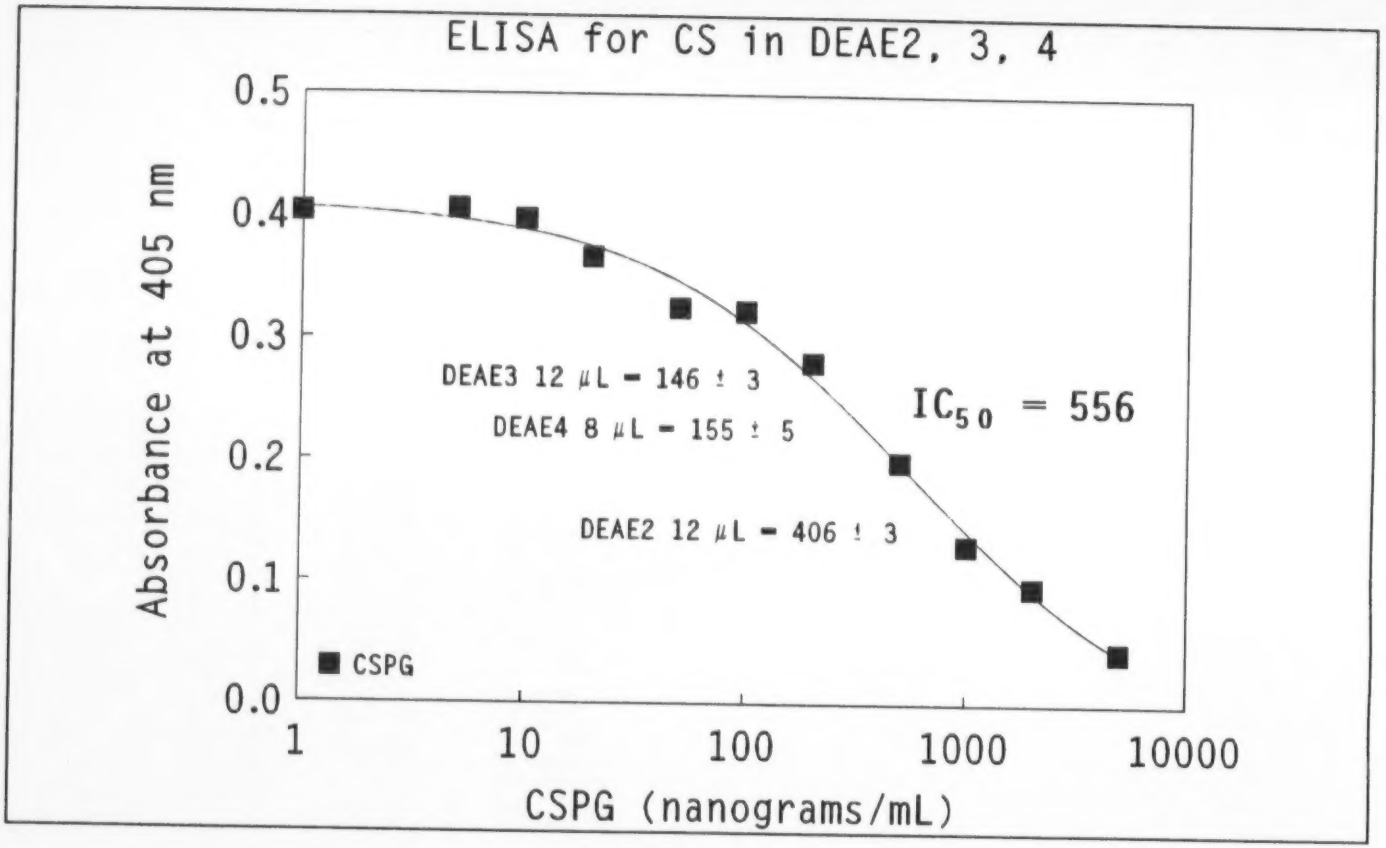


Figure IV.2.1.B.
ELISA for CS in DEAE 2,3,4

CSPG from adult bovine TMJ articular disc was used to coat the ELISA plate as well as for the antigenic standards. Antibody CS56 was used at a dilution of 1:10 000 in PBST. DEAE 2, 3 and 4 were duplicated with a mean values of 406 ± 3, 146 ± 3 and 155 ± 5 respectively.

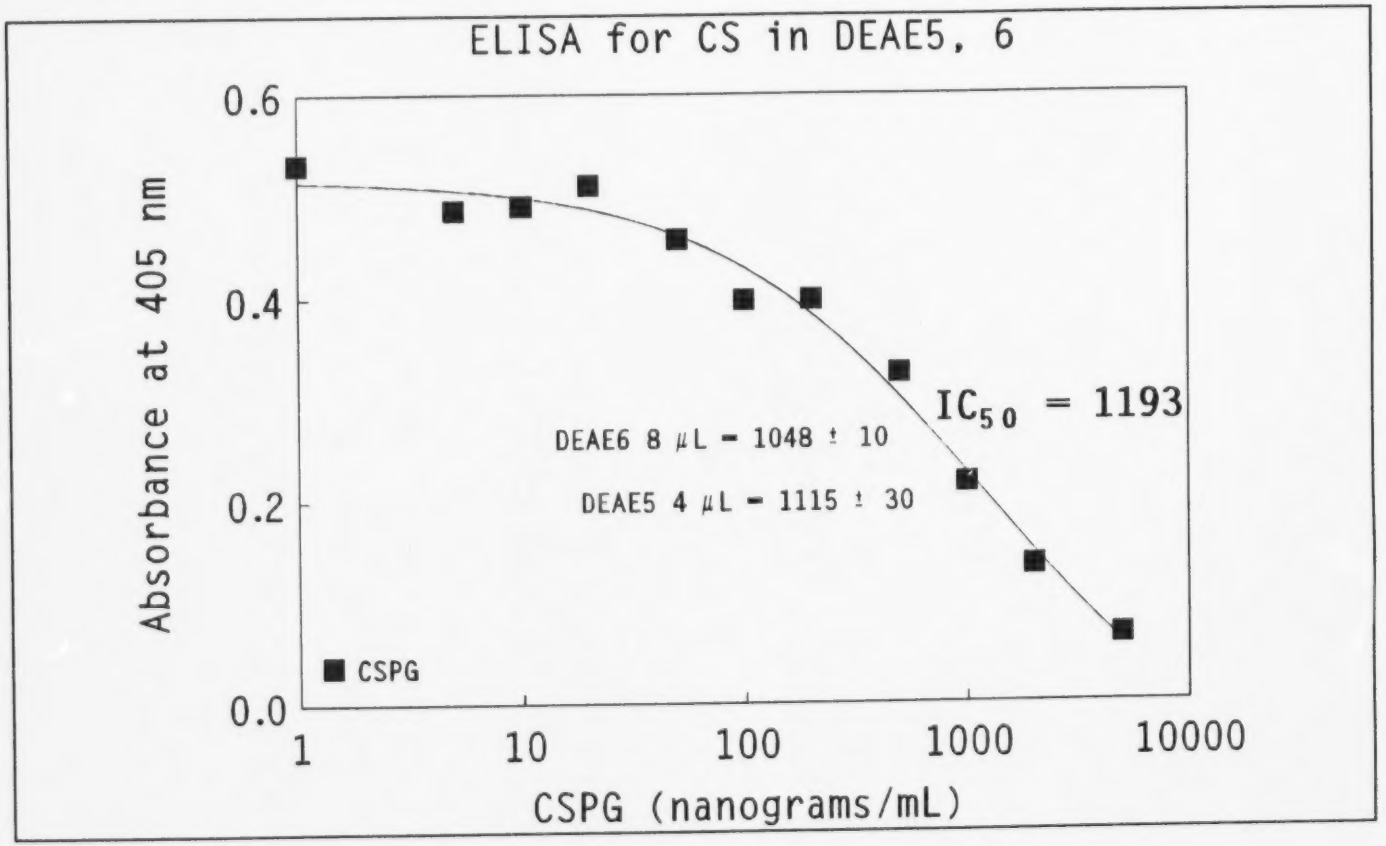


Figure IV.2.1.C.

ELISA for CS in DEAE 5, 6

CSPG from adult bovine TMJ articular disc was used to coat the ELISA plate as well as for the antigenic standards. Antibody CS56 was used at a dilution of 1:10 000 in PBST. DEAE 5 and 6 were duplicated with a mean values of 1115 ± 30 and 1048 ± 10 respectively.

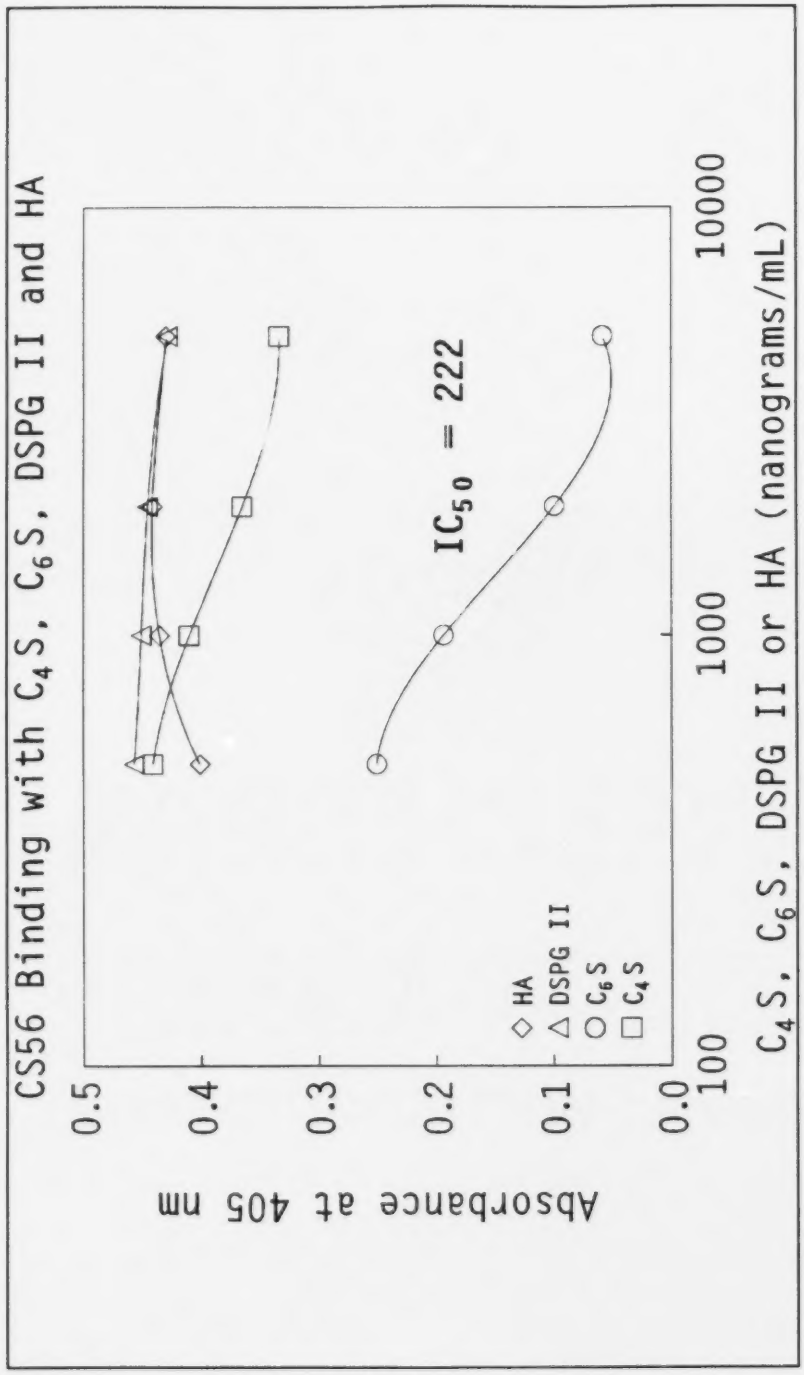


Figure IV.2.1.D.
ELISA for C_4S, C_6S, DSPG II and HA

CSPG from adult bovine TMJ articular disc was used to coat the ELISA plate. The C_4S standard was from whale cartilage containing (according to Seikagaku®) C_4S and C_6S in a ratio of 80:20. The C_6S antigenic standard was from shark cartilage containing (according to Seikagaku®) C_4S and C_6S in a ratio of 10:90. DSPG II was from adult bovine skin. HA was from Sigma®. Antibody CS56 was used at a dilution of 1:10 000 in PBST.

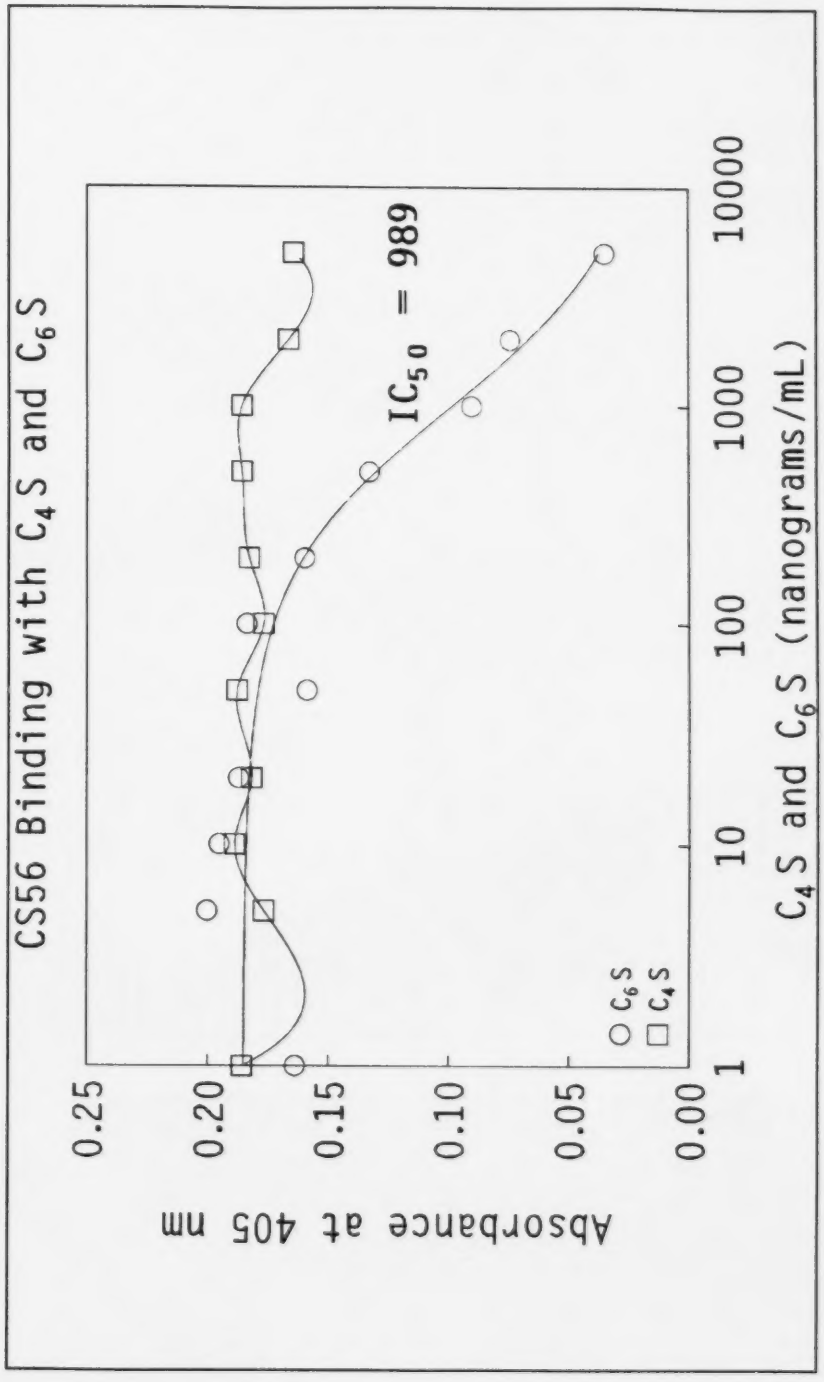


Figure IV.2.1.E.
ELISA for C_4S, and C_6S

CSPG from adult bovine TMJ articular disc was used to coat the ELISA plate. The C_4S standard was from whale cartilage containing (according to Seikagaku[3]) C_4S and C_6S in a ratio of 80:20. The C_6S antigenic standard was from shark cartilage containing (according to Seikagaku[3]) C_4S and C_6S in a ratio of 10:90. Antibody CS56 was used at a dilution of 1:10 000.

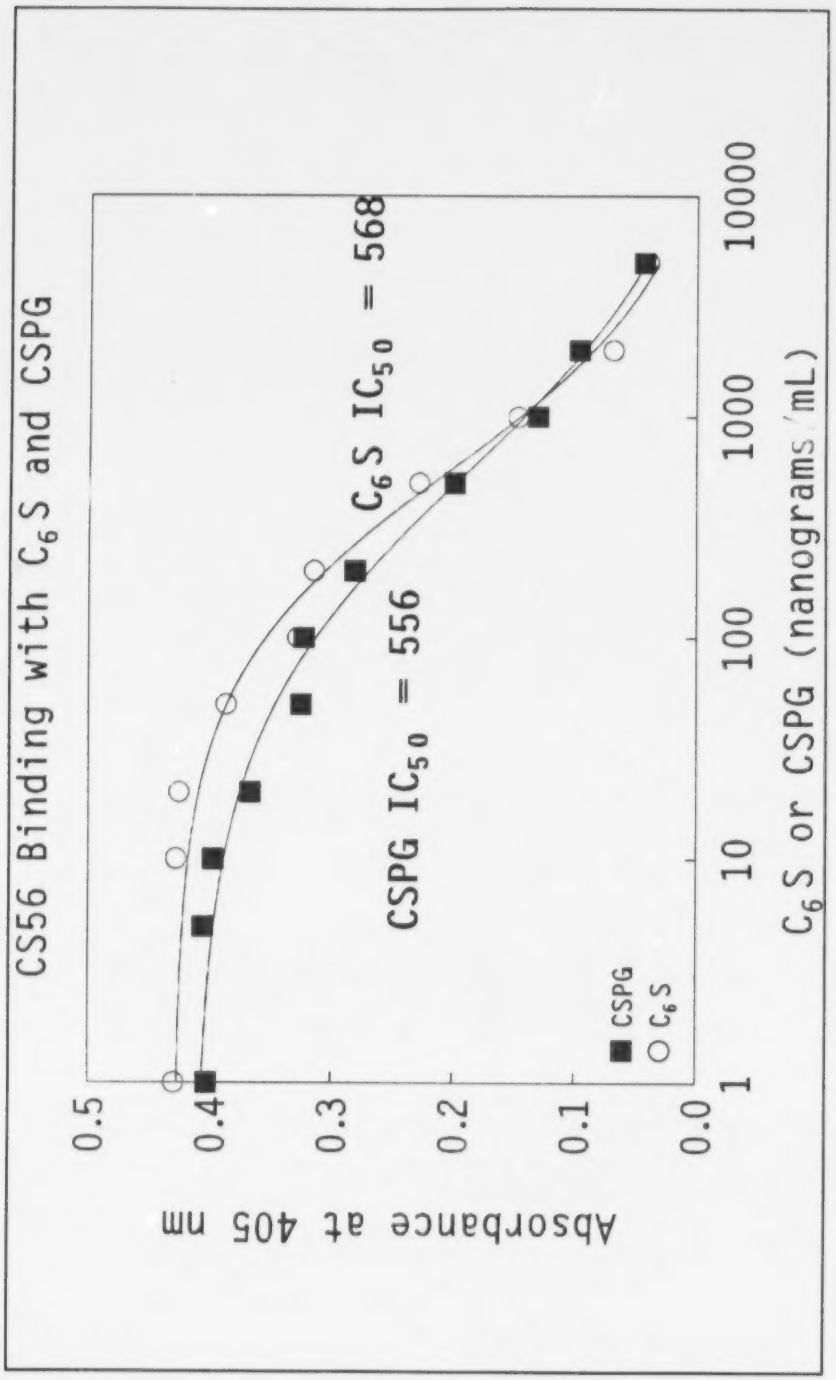


Figure IV.2.1.F.
ELISA for C_6S and CSPG (First Trial)
CSPG from adult bovine TMJ articular disc was used to coat the ELISA plate as well as for the CSPG antigenic standards. The C_6S antigenic standard was from shark cartilage (Seikagaku[3]). Antibody CS56 was used at a dilution of 1:10 000 in PBST.

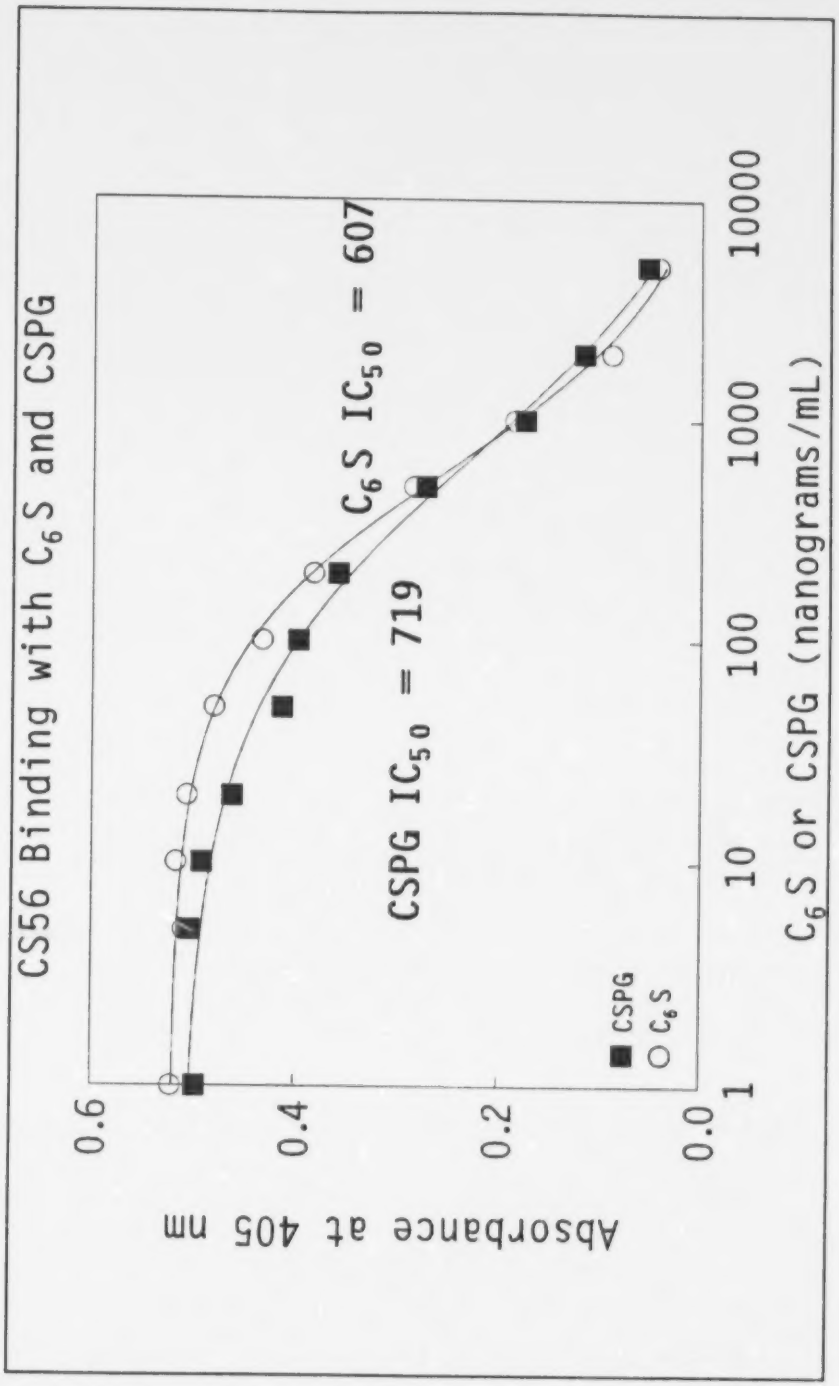


Figure IV.2.1.G.
ELISA for C_6S and CSPG (Second Trial)
CSPG from adult bovine TMJ articular disc was used to coat the ELISA plate as well as for the CSPG antigenic standards. The C_6S antigenic standard was from shark cartilage (Seikagaku®). Antibody CS56 was used at a dilution of 1:10 000 in PBST.

2.2. Dermatan Sulphate Proteoglycan II

Monoclonal antibody 6D6 was used at a concentration of 1:100 for the ELISA's for DSPG II (PDS). Assays were performed for DSPG II on all the column collections (Figure IV.2.2.A.,B.,C.). as well as on a sample of untreated fetal calf serum (Figure IV.2.2.D.). The fetal calf serum (FCS) was simply taken out of the bottle Gibco® (Lot # 50K9406) and mixed with twice concentrated PBST. Results for the DEAE pools are listed in Table IV.2.2.A.

DEAE1	DEAE2	DEAE3	DEAE4	DEAE5	DEAE6
147 μg	154 μg	55 μg	74 μg	131 μg	164 μg

Table IV.2.2.A.
DSPG II/100 ml Culture Medium

The untreated fetal calf serum was found to contain 5.6 μg DSPG II/ml. Therefore, in 220 ml of medium containing 2% FCS, there would be 25 μg of DSPG II with 11.4 μg/100 ml of medium.

The average quantity of DSPG II in conditioned medium containing 2% FCS (DEAE1, 2, 5 and 6) was calculated to be 150 ± 19 μg/100 ml of unconcentrated medium. The unconditioned medium containing 2% FCS (DEAE4) was found to contain approximately one-half of the amount of DSPG II as in conditioned medium containing 2 % FCS (74 μg/100 ml). The conditioned medium with 0% FCS (DEAE3) was found to contain about one-third of that of conditioned medium with 2% FCS.

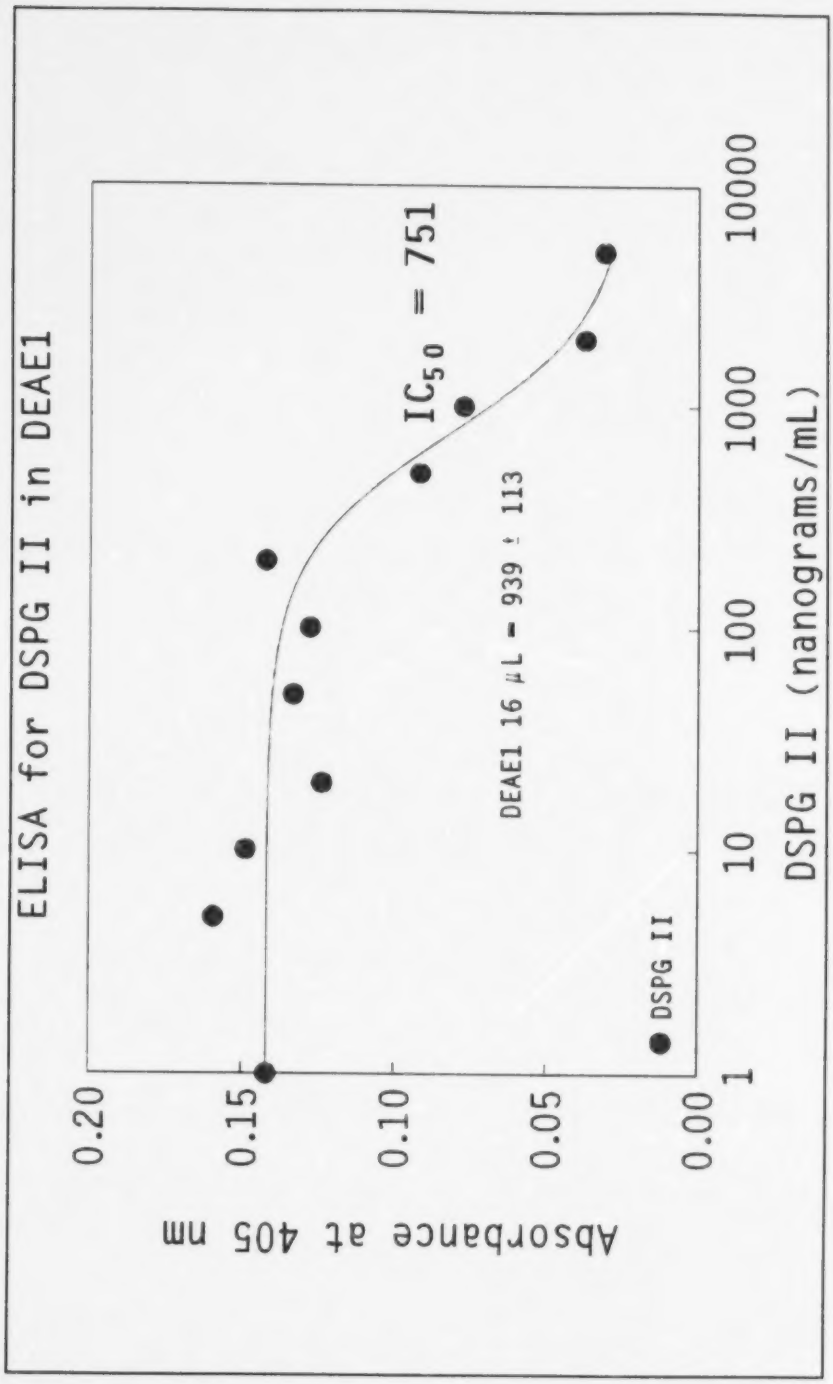


Figure IV.2.2.A.
ELISA for DSPG II in DEAE1
DSPG II from adult bovine skin was used to coat the ELISA plate as well as for the antigenic standards. Antibody 6D6 was used at a dilution of 1:100 in PBST. The DEAE1 sample was duplicated with a mean value of 939 ± 113.

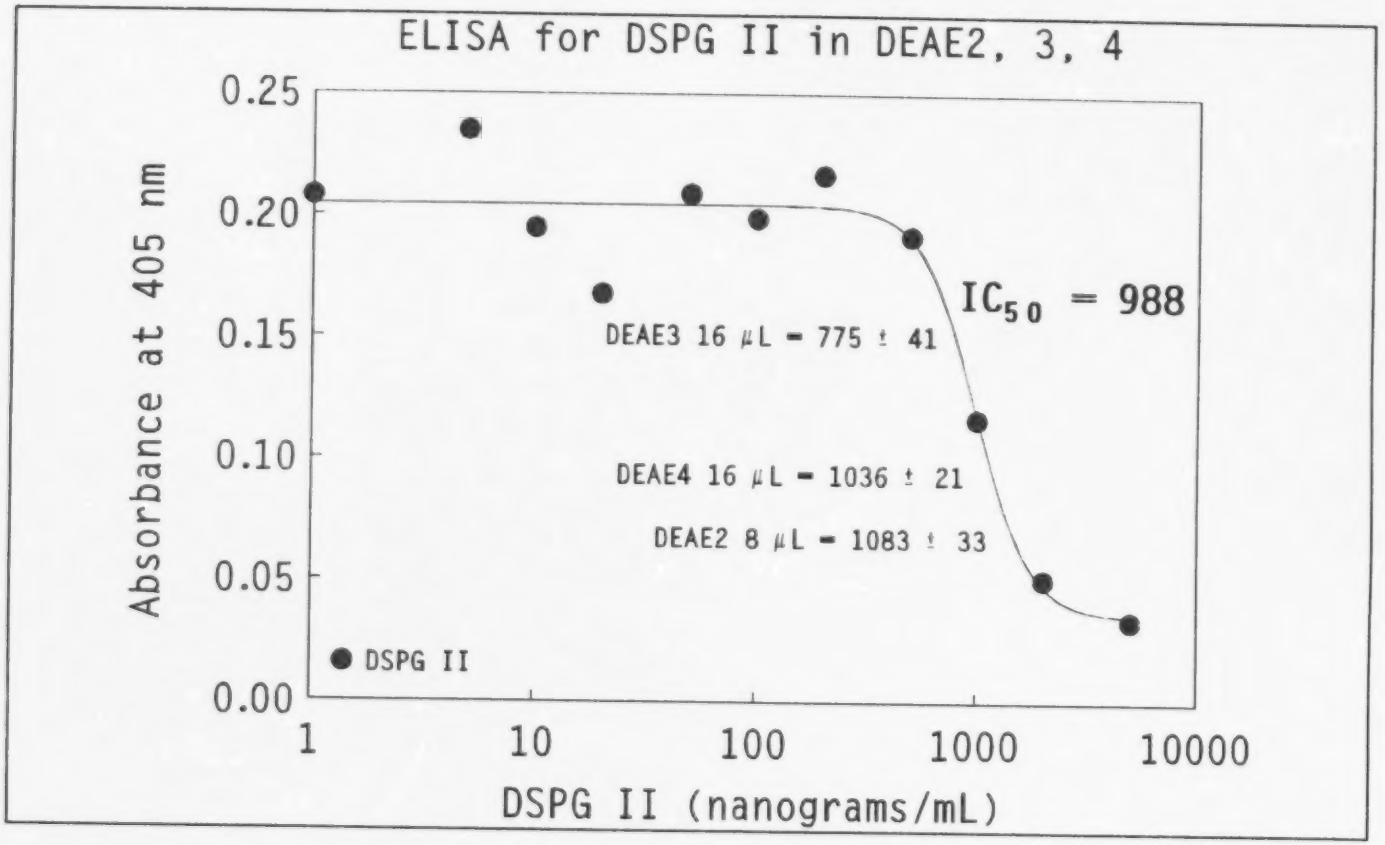


Figure IV.2.2.B.

ELISA for DSPG II in DEAE 2,3,4

DSPG II from adult bovine skin was used to coat the ELISA plate as well as for the antigenic standards. Antibody 6D6 was used at a dilution of 1:100 in PBST. DEAE 2, 3 and 4 samples were duplicated with a mean values of 1083 ± 33, 775 ± 41 and 1036 ± 21 respectively.

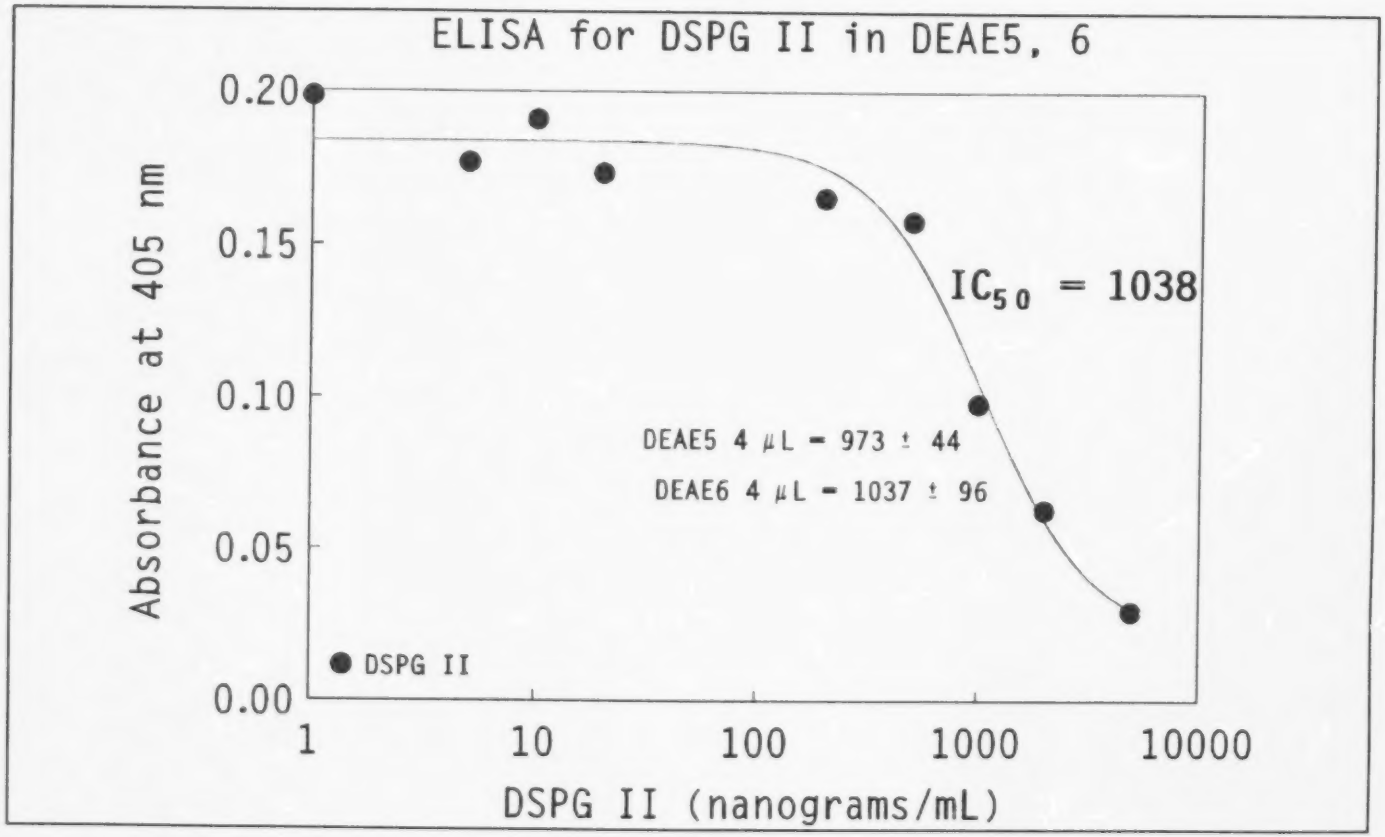


Figure IV.2.2.C.

ELISA for DSPG II in DEAE 5 and 6

DSPG II from adult bovine skin was used to coat the ELISA plate as well as for the antigenic standards. Antibody 6D6 was used at a dilution of 1:100 in PBST. DEAE 5 and 6 samples were duplicated with a mean values of 973 ± 44 and 1037 ± 96 respectively.

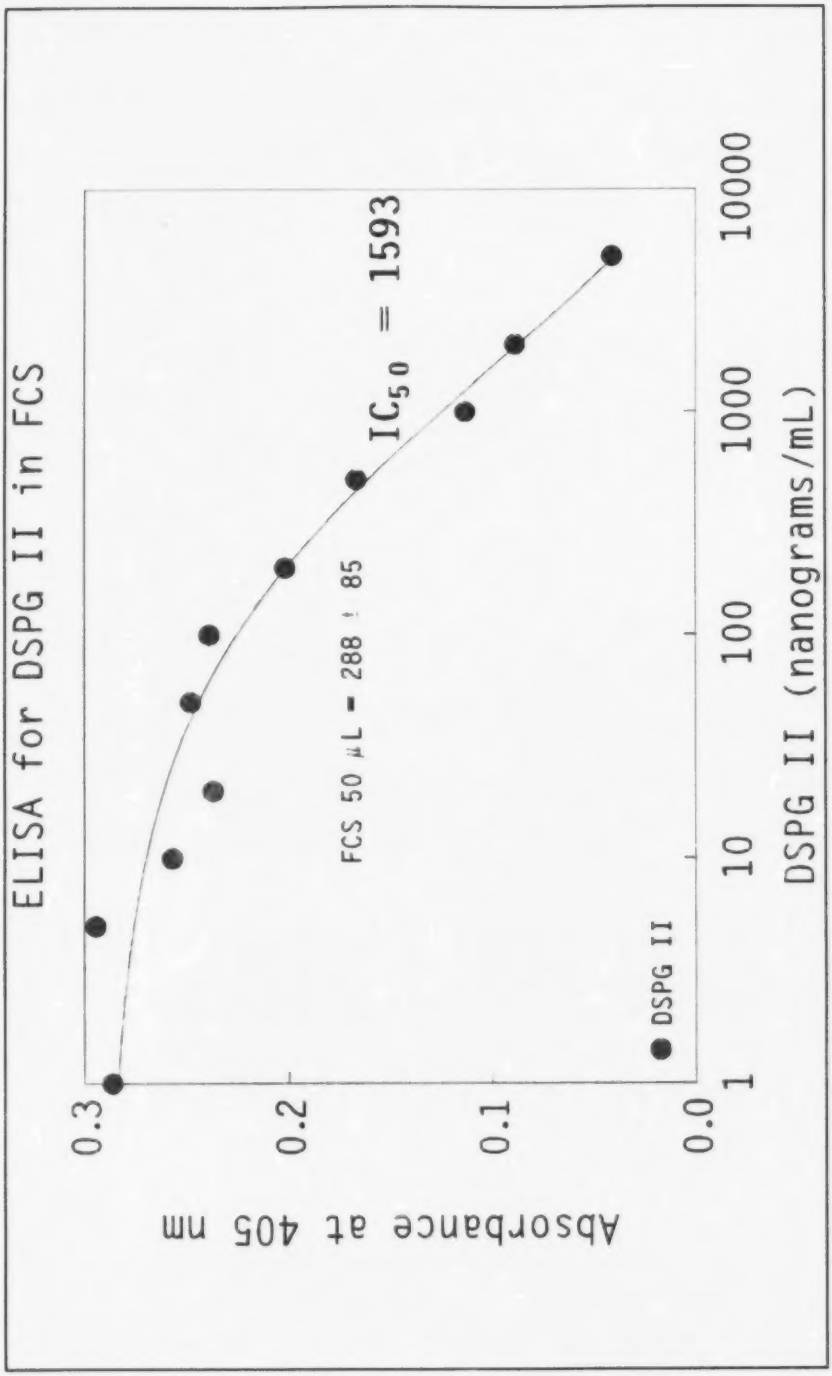


Figure IV.2.2.D.
ELISA for DSPG II in FCS
DSPG II from adult bovine skin was used to coat the ELISA plate as well as for the antigenic standards. Antibody 6D6 was used at a dilution of 1:100 in PBST. The FCS sample was duplicated with a mean value of 288 ± 85.

2.3. Keratan Sulphate

Keratan sulphate was assayed for in DEAE 5 using the monoclonal antibody AH12 at a dilution of 1:8000. The amount of KS found in the sample was 13 μg/100 ml of cell culture medium (Figure IV.2.3.A.).

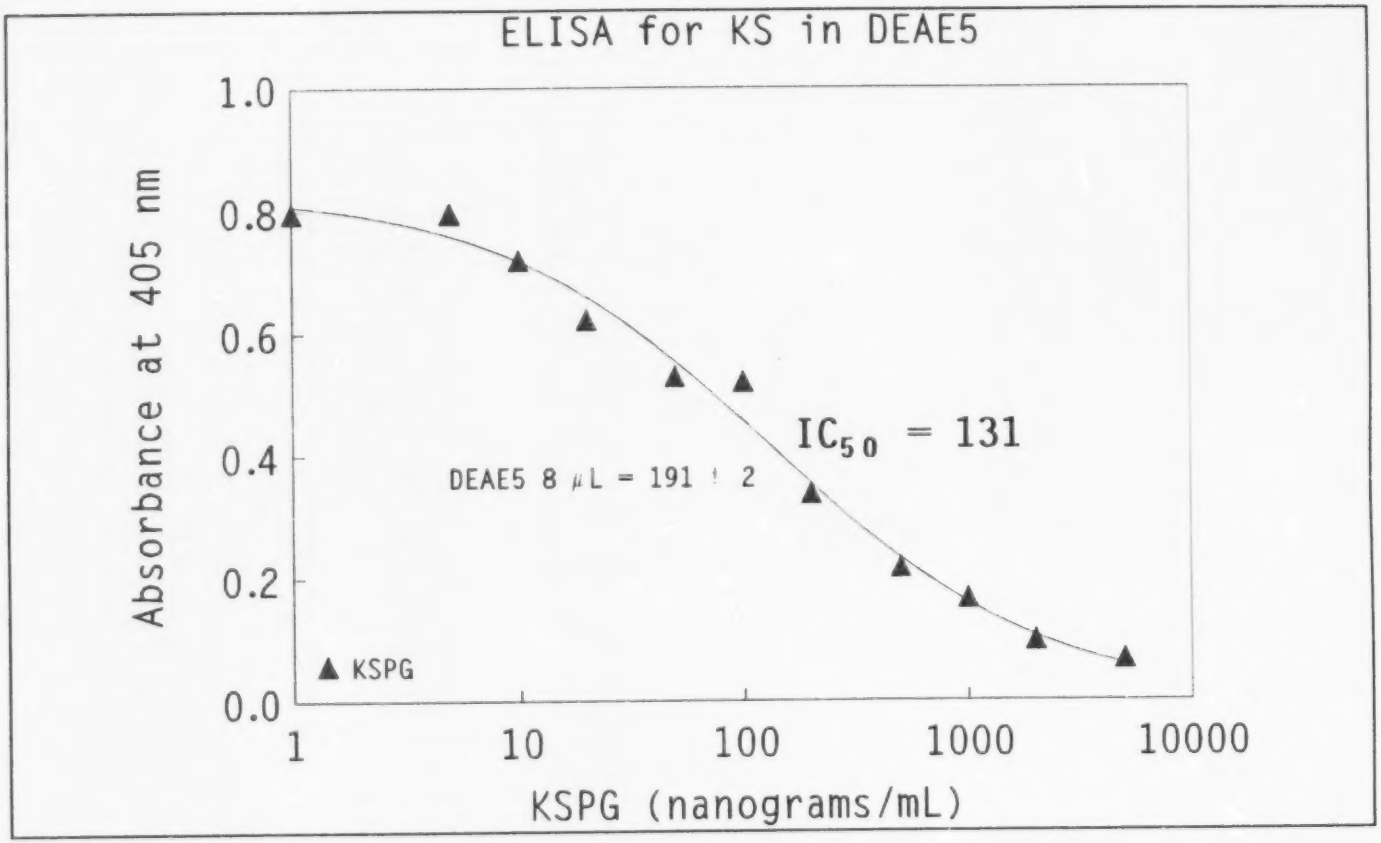


Figure IV.2.3.A.

ELISA for KSPG in DEAE 5

CSPG from adult bovine TMJ articular disc was used to coat the ELISA plate. KS antigenic standards were from adult bovine TMJ articular disc. Antibody AH12 was used at a dilution of 1:8000 in PBST. The DEAE 5 sample was duplicated with a mean value of 191 ± 2.

3. Uronic Acid Analysis

3.1. Uronic Acid Content

Uronic acid was analyzed on all the DEAE column pools using the method described by Kosakai and Yosizawa (1979). All trials were performed with replicate samples. Results are presented in Table IV.3.1.A. Values are expressed in micrograms of uronic acid per 100 ml of original cell culture medium.

DEAE1	DEAE2	DEAE3	DEAE4	DEAE5	DEAE6
70 μg	26 μg	20 μg	17 μg	76 μg	80 μg

Table IV.3.1.A.
Uronic Acid/100 ml of Unconcentrated Culture Medium

The advantage of this method over that of Bitter and Muir (1962) is its improved color development to iduronic acid. This result was tested utilizing a DS standard (from hog skin) which revealed that it was 29.2% uronic acid. This result is slightly lower than the 36.8% reported by Kosakai and Yosizawa (1979), but their data was based on a dermatan sulphate standard that was prepared in their laboratory.

4. Iduronic Acid Analysis

4.1. Iduronic Acid Content

The Di Ferrante method is a colorimetric measurement of dermatan sulphate. This technique allows for the direct measurement of dermatan sulphate in a mixture of glycosaminoglycans (Di Ferrante *et al.*, 1971).

A Di Ferrante analysis was performed on column material from all DEAE fractions. As well, C_4S and C_6S standards were tested. No color developed with either of these standards. The values for dermatan sulphate were then multiplied by 0.292 (from uronic acid analysis of DS standard) to determine the uronic acid values. Since the proportion in total uronic acid is approximately 80% (Gibson and Pearson, 1982), the value for iduronic acid was multiplied by 0.8 to obtain the uronic acid content. Therefore, the formula used was: **IdU.A. =[DS] x 0.292 x 0.8**

This value was then compared to the earlier uronic acid measurement. As before, values are expressed as micrograms per 100 ml of unconcentrated cell culture medium.

DEAE #	IdU.A. μg/100 ml	DS μg/100 ml	U.A.μg/100 ml
1	64	274	80
2	66	282	82
3	28	119	35
4	25	105	31
5	19	83	24
6	33	140	41

Table IV.4.1.A.
Iduronic Acid/100 ml Unconcentrated Cell Culture Medium

5. Cellulose Acetate Electrophoresis

5.1. GAG Representation

Following digestion with papain, cellulose acetate electrophoresis was performed on all DEAE column collections. Initially a phosphate buffer system was used and although this system was able to separate HA from the other GAGs, it did not provide separation of CS from DS (Figure IV.5.1.A.). Two peaks were observed for the standard on the electrophoretogram; a smaller initial HA peak followed by a much larger second peak representing CS and DS. The standard used here and in all following runs contained 1.4 μg each of HA, CS and DS per microliter.

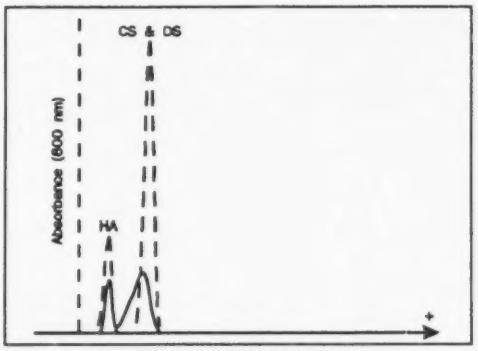


Figure IV.5.1.A.
DEAE1 (Phosphate Buffer)
Standard (Dashed), DEAE1 (Solid)

The DEAE1 sample exhibited two peaks as well. The first peak showed electophoretic mobility similar to that of the standard HA peak. Although electophoretic mobility is similar, at this time, it cannot be said that this peak represents HA. (In subsequent trials using *Streptomyces* hyaluronidase, this result was further tested.) The second peak appeared to be slightly less mobile and broader than the standard CS/DS peak and again, it is difficult to draw any conclusions here based upon electrophoretic mobility alone.

Relative proportions of the GAGs in the DEAE1 sample were approximated by photocopying the chart strip and cutting out and weighing the peaks on a microbalance. Using this method, it was determined that HA comprised 37% of the total GAGs in the DEAE1 sample. Since HA is non-sulphated, it does not stain as intensely with Alcian blue as the other GAGs which are sulphated. Therefore, a compensation factor was determined by comparing the size of the HA peak to the CS/DS peak in the standard. The weight of the HA peak was divided by the one-half the weight of the CS/DS peak to obtain the compensation factor. This factor was then used to calculate the proportion of HA in the DEAE sample.

To verify that the initial peak was HA, a *Streptomyces* hyaluronidase digestion was performed on the DEAE1 sample (Figure IV.5.1.B.). Following enzymatic treatment, the initial peak was removed, providing further evidence that this peak represents HA.

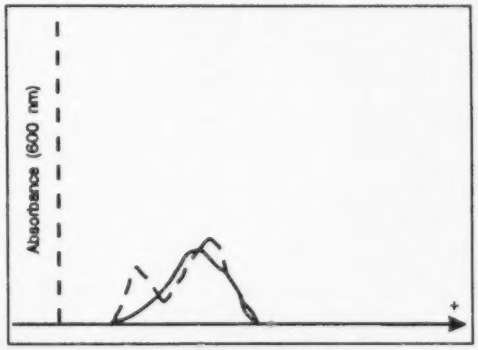


Figure IV.5.1.B.
DEAE1 with *S.* Hyaluronidase
Untreated (Dashed), Enzyme (Solid)

An experiment was conducted to determine if there was a significant contribution of HA from the fetal calf serum (Figure IV.5.1.C.). Serum was taken straight from the bottle and treated in the same manner with papain. Following ethanol

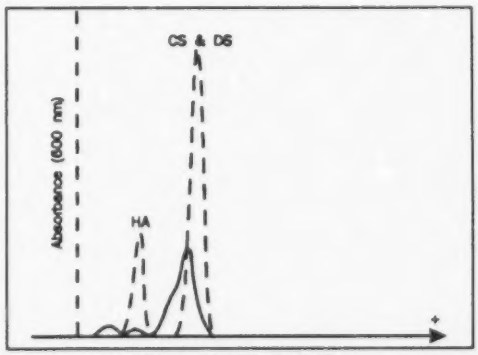


Figure IV.5.1.C.
FCS
Standard (Dashed), FCS (Solid)

precipitation, the sample was resuspended in water and electrophoresed. There appeared to be at least three peaks in the FCS sample; the first one was very small and appeared earlier than the HA standard and may have possibly been heparan sulphate. The second one was very small as well and appeared to have the same mobility as HA. The last one was the largest and occurred with average mobility slightly slower than the CS/DS peak. It is difficult to comment on the composition of this peak as the GAG composition of serum is not well established.

A pyridine-acetate buffer system was used with the remainder of the cellulose acetate trials as this system provided better separation of CS and DS (Figure IV.5.1.D.). Three discrete peaks were observed in the standard; the first one represented HA, followed by DS and lastly CS. Although this method was able to separate CS from DS, only two peaks were observed in the DEAE1

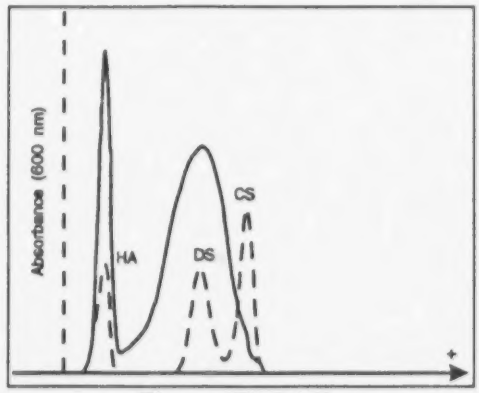


Figure IV.5.1.D.
DEAE1 (Pyridine-Acetate Buffer)
Standard (Dashed), DEAE1 (Solid)

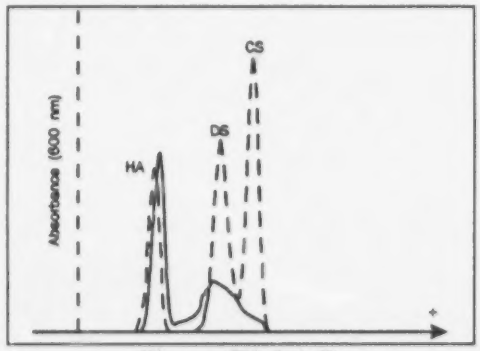


Figure IV.5.1.E.
DEAE2
Standard (Dashed), DEAE2 (Solid)

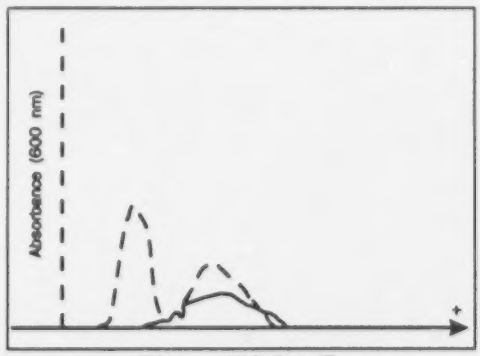


Figure IV.5.1.F.
DEAE2 with *S.* Hyaluronidase
Untreated (Dashed), Enzyme (Solid)

sample. Using the same method as above with cutting and weighing the peaks, the HA content was calculated to be 33% of the total GAGs in the sample.

Two peaks were also observed in DEAE2 as well (Figure IV.5.1.E). Unlike the previous sample, the first peak is larger than the second one. Furthermore, the mobility of this peak seemed to correspond to that of the HA standard. Following treatment with *Streptomyces* hyaluronidase, this peak was obliterated (Figure IV.5.1.F). Using the same methods as above, HA was calculated to comprise 30% of the total GAGs in DEAE2.

To test the efficacy of the *Streptomyces* hyaluronidase enzyme in the pyridine-acetate buffer system, the control standard of 1 μg/μl of HA, CS and DS was treated with the enzyme (Figure IV.5.1.G). Following this treatment, an HA peak was not detectable.

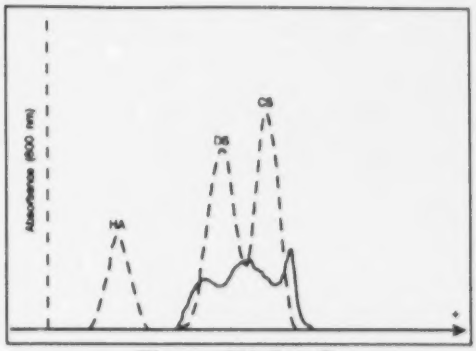


Figure IV.5.1.G.
Standard with *S.*Hyaluronidase
Untreated (Dashed), Enzyme (Solid)

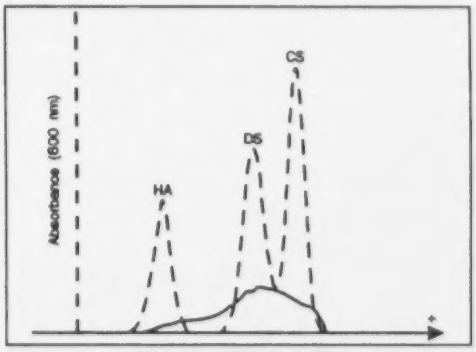


Figure IV.5.1.H.
DEAE3
Standard (Dashed), DEAE3 (Solid)

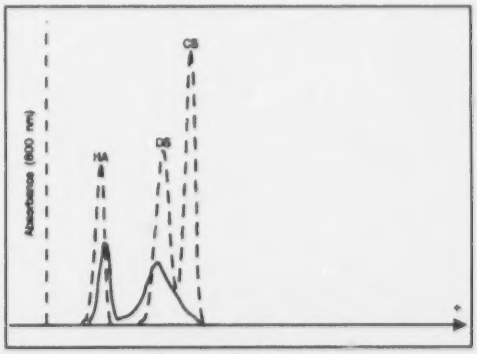


Figure IV.5.1.I.
DEAE4
Standard (Dashed), DEAE4 (Solid)

DEAE3 exhibited an indistinct broad band with the major portion of the peak occurring at the same position as CS and DS (Figure IV.5.1.H). There was only a very slight rise in the area of the HA standard. It is uncertain whether there was a significant amount of HA in this sample.

DEAE4 displayed two peaks with the first one being slightly higher and having mobility similar to the HA standard (Figure IV.5.1.I). The second one was lower but relatively broader with an average mobility close to that of the DS standard. To confirm that the first peak was HA, a digestion with *Streptomyces* hyaluronidase was performed to remove this peak (Figure IV.5.1.J). In contrast to the earlier trial with untreated fetal calf serum, this column collection of unconditioned cell culture medium appeared to contain a significant amount of HA. This result was not surprising as serum was expected to contain amounts of HA.

Figure IV.5.1.J.
DEAE4 with *S.* Hyaluronidase
Untreated (Dashed), Enzyme (Solid)

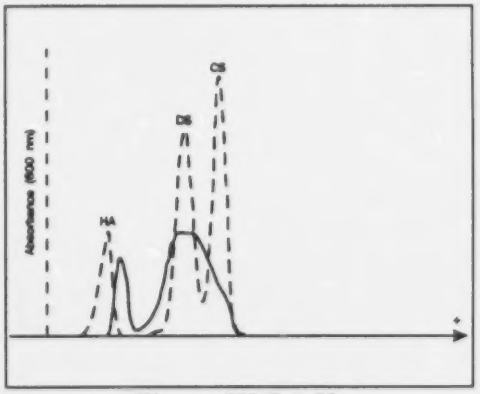


Figure IV.5.1.K.
DEAE5
Standard (Dashed), DEAE5 (Solid)

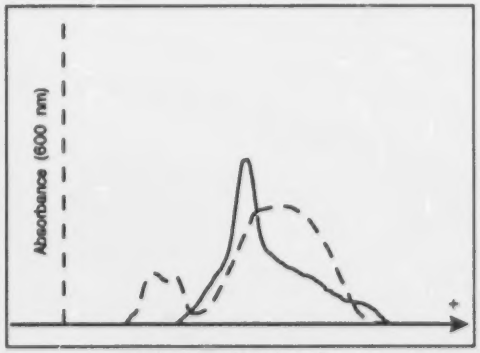


Figure IV.5.1.L.
DEAE5 with *S.* Hyaluronidase
Untreated (Dashed), Enzyme (Solid)

DEAE5 exhibited two peaks as well (Figure IV.5.1.K). The initial peak was in the approximate area of the HA standard peak and the second one was broader with its apex in the area of the DS standard and its base extending across both CS and DS peaks. HA content was calculated to be approximately 33% of the total GAGs in the DEAE sample. As before, to verify that this initial peak was HA, a digest with *Streptomyces* hyaluronidase was performed (Figure IV.5.1.L.). These results are consistent with the earlier DEAE1 and 2 pools which were derived from smaller volumes of medium containing 2% FCS.

To reveal if DS was a significant component of the DEAE collection, a digestion was performed with chondroitinase B. The enzyme was first tested with a DS standard (1.4 μg) (Figure IV.5.1.M.). The enzyme appeared to be very efficient as there was no detectable DS peak following treatment. Following digestion with chondroitinase B,

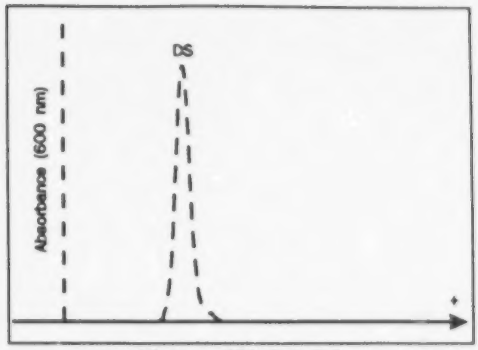


Figure IV.5.1.M.
DS with Chondroitinase B
Untreated (Dashed), Enzyme (Solid)

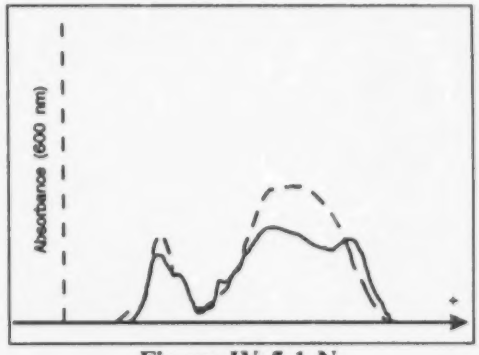


Figure IV.5.1.N.
DEAE5 with Chondroitinase B
Untreated (Dashed), Enzyme (Solid)

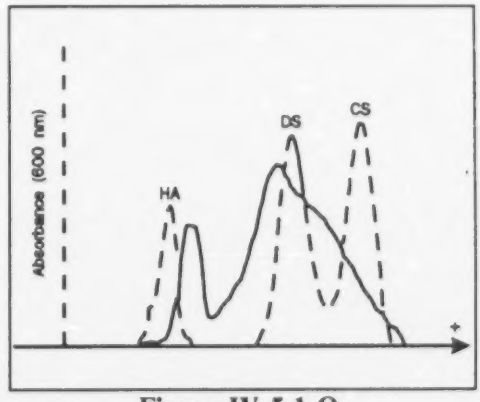


Figure IV.5.1.O.
DEAE6
Standard (Dashed), DEAE6 (Solid)

the second peak of DEAE5 was reduced by approximately one third (Figure IV.5.1.N.). This suggested that there were amounts of DS in the DEAE collection.

Sample DEAE6 displayed two peaks as well, with the first one roughly corresponding to the HA peak of the standard with almost the same position and size and the second larger peak apexing in the area of the DS peak and extending its base beyond both CS and DS standards(Figure IV.5.1.O). The HA content of the sample was calculated to be 32% of the total GAGs in the sample. The HA pattern and percentage of total GAGs are in agreement with the results obtained for DEAE1,2, and 5.

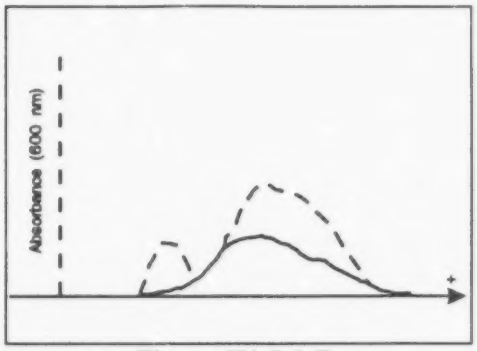


Figure IV.5.1.P.
DEAE6 with *S.* Hyaluronidase
Untreated (Dashed), Enzyme (Solid)

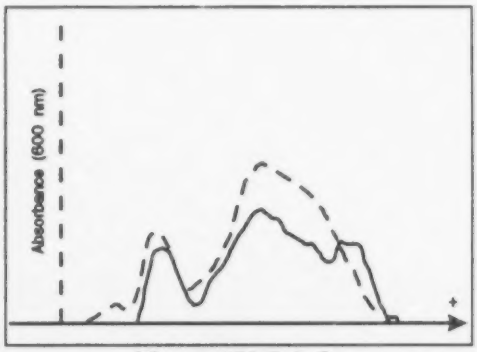


Figure IV.5.1.Q.
DEAE6 with Chondroitinase B
Untreated (Dashed), Enzyme (Solid)

As before, a digestion with *Streptomyces* hyaluronidase was performed to abolish the first peak and verify that it was due to HA in the sample (Figure IV.5.1.P.).

Following treatment with Chondroitinase B, the second larger peak was again reduced by approximately one third (Figure IV.5.1.Q). This result is consistent with that of the DEAE5 pool.

In summary, it appears that HA constitutes approximately one third of the total

GAGs in DEAE1, 2, 5 and 6. These DEAE pools were all conditioned medium with 2% fetal calf serum. DEAE3 was conditioned medium with no FCS and did not appear to contain a significant amount of HA (Figure IV.5.1.H). DEAE4 was unconditioned medium with 2% FCS and appeared to contain a significant amount of HA. This result was not surprising as serum was expected to contain HA. Based on these results, it was difficult to ascertain the contribution of HA from the serum versus the amount produced by the cultured cells.

Lastly chondroitinase B produced a reduction in the second peak by approximately one third, suggesting that there was DS in DEAE5 and 6.

6. Thin Layer Chromatography

6.1. Chondroitin Sulphate Representation

Thin layer chromatography was performed to determine the relative amounts of C_4S and C_6S in the DEAE collections. Following papain and chondroitinase ABC digestion, the DEAE column products were assayed with this technique. An approximate amount of 5 and 10 micrograms of CS (calculated from the uronic acid analysis) from DEAE1 (10 μl and 20 μl of sample respectively) were tested with 8 micrograms (2 μl) each of ΔDi-6S, ΔDi-4S and ΔDi-0S (Figure IV.6.1.A). A lane was run with 8 μg of each applied one on top of another to verify that separation of these standards was possible. Lastly 20 μl of buffer was tested to determine if it produced any artifacts.

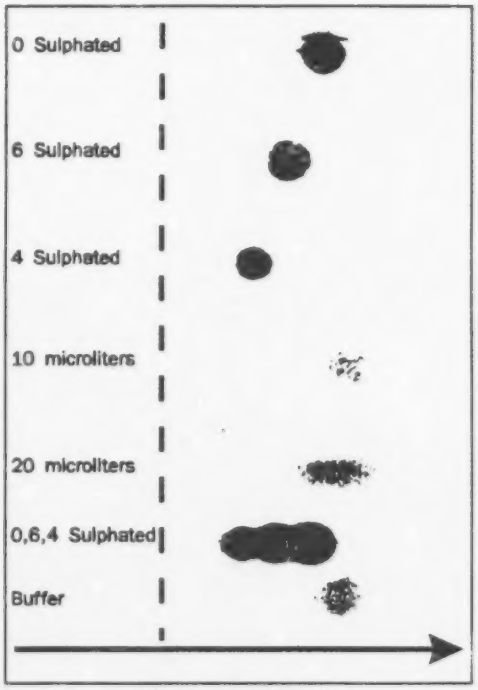


Figure IV.6.1.A.
DEAE1
Thin Layer Chromatography

The results indicated that this method was effective in separating the disaccharide standards, however the results for the DEAE1 sample were questionable. The standards presented as dark purple spots with clearly demarcated margins, while the DEAE sample and the buffer appeared as diffuse purplish-pink areas. Although there appeared to be

some color development in both the 10 and 20 μl lanes, the position of this color seemed to correspond to that of the buffer. In the 20 μl lane, there was some material with the similar mobility as ΔDi-0S, however it was uncertain if this was due to a non-sulphated GAG such as HA disaccharide or if it was merely a "trail" of buffer.

Thin layer chromatography was repeated with papain and chondroitinase ABC treated column material from DEAE5 and 6. Based on the uronic acid analysis, approximately 12 μg (30 μl) of sample was spotted for each lane. The same standards were spotted as well as 20 μl of buffer. Unfortunately, results were nearly identical to that of DEAE1. The standards behaved in the same manner as well as the buffer. Again, there was a slight haze corresponding to the ΔDi-0S standard, but there appeared to be the same interference from the buffer.

7. Gel Electrophoresis and Immunoblotting

7.1. Polyacrylamide Gel Electrophoresis

Proteoglycans from DEAE5 and 6 were analyzed with SDS gel electrophoresis (5% polyacrylamide) following enzymatic digestion with chondroitinase ABC. The standards used were adult bovine skin DSPG II and CSPG from adult bovine TMJ articular disc. Twenty micrograms of DSPG II and CSPG were used per lane, while 220 μg of DEAE5 and 6 were placed per lane. All lanes were duplicated with the first one left untreated while the second one was treated with chondroitinase ABC (Plate IV.7.1.A.).

On the Stains-All stained gel, CSPG exhibited very minimal mobility due to its large molecular size. The similar mobility of DEAE5 and 6 suggested that their proteoglycans also had a very large molecular size as they were only slightly able to penetrate the gel.

The Coomassie blue stained gel showed that the chondroitinase ABC produced cores of DEAE5 and 6 were extremely large as they barely penetrated the 5% gel. Although molecular weight standards were used, the size of these cores was difficult to judge as they were off the scale. However an estimate of greater than 200 kDa would not seem unreasonable.

Plate IV.7.1.A.

Gel Electrophoresis and Immunoblotting of DEAE5 and 6

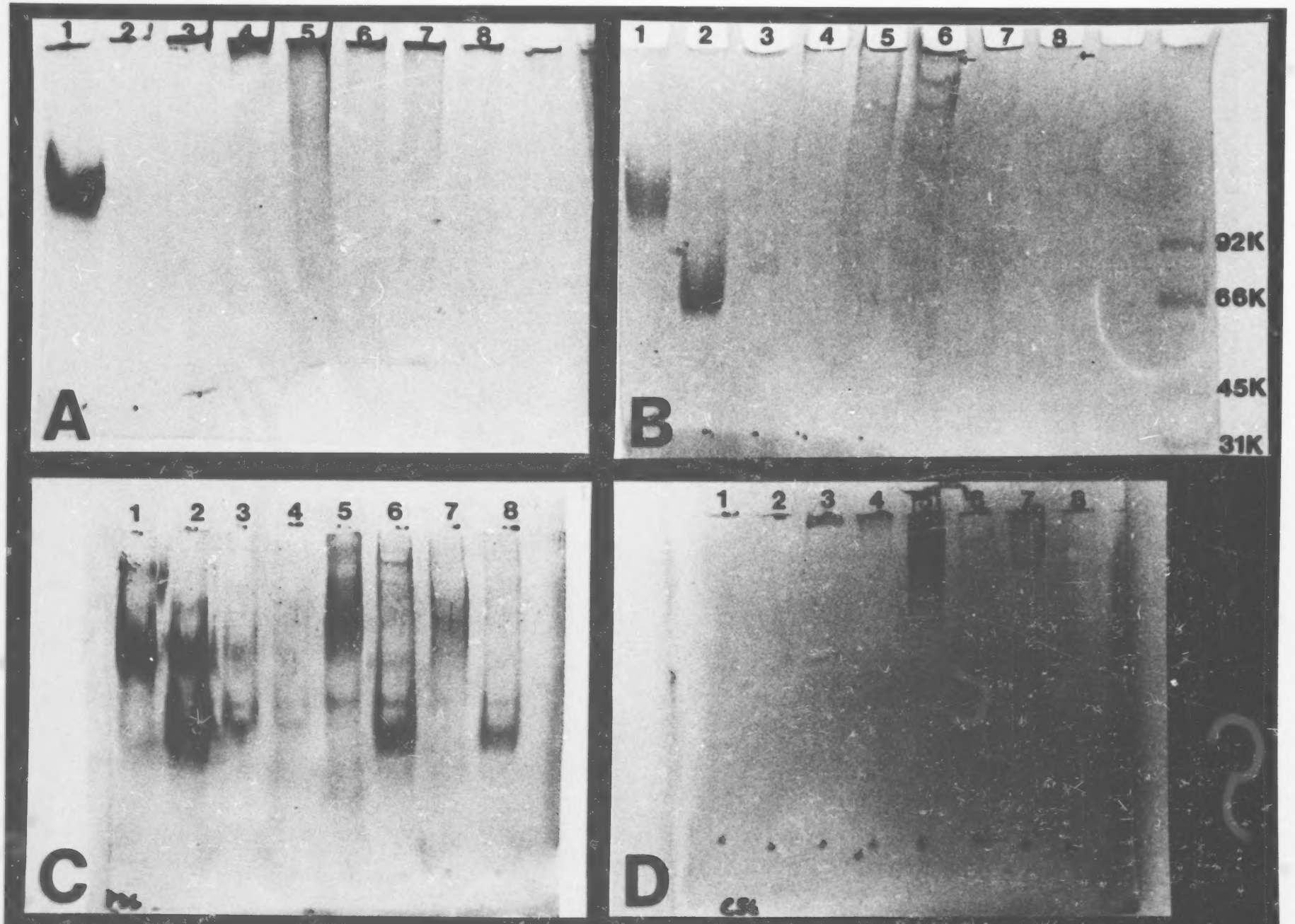
Lane Arrangement for A,B,C and D: Lane 1- skin DSPG II, Lane 2 - skin DSPG II treated with Chondroitinase ABC, Lane 3 - CSPG, Lane 4 - CSPG treated with Chondroitinase ABC, Lane 5 - DEAE5, Lane 6 - DEAE5 treated with Chondroitinase ABC, Lane 7 - DEAE6, Lane 8 - DEAE6 treated with Chondroitinase ABC

A: Stains-All Stain

B: Coomassie Blue Stain with the addition of Lane 10 - Molecular Weight Standards (in descending order of molecular weight): phosphorylase B (92K), bovine serum albumin (66K), ovalbumin (45K), and carbonic anhydrase (31K). Arrows indicate location of Chondroitinase ABC produced cores.

C: DSPG II Stain using Antibody 6D6

D: CS Stain using Antibody CS56


1 2 3 4 5 6 7 8
A
1 2 3 4 5 6 7 8
92K
66K
45K
31K
B
1 2 3 4 5 6 7 8
C
1 2 3 4 5 6 7 8
D

7.2. Immunoblotting

The gel arrangement for immunoblotting was identical to that of above. Smaller quantities were used as 2 μg of DSPG II, 5 μg of CSPG, 100μg of DEAE5 and 6 were placed in the respective lanes (Plate IV.7.1.A.).

Staining with antibody 6D6 suggested that there was DSPG II in both DEAE5 and 6. In both of these samples, there was a stained band which corresponded to that of the DSPG II standard.

Antibody CS56 (αCS) revealed staining of the untreated DEAE samples, however, this staining was not apparent with the samples that were treated with chondroitinase ABC. This result strongly suggested that there was CS in the DEAE pools. A slight amount of staining was observed with the untreated CSPG standard while this was not observed with chondroitinase ABC treated standard. No staining was observed in either of the DSPG II standards.

CHAPTER FIVE

DISCUSSION

DISCUSSION

OUTLINE

1. Microscopy of the Fetal TMJ Articular Disc 170

1.1. Histology 170

1.2. Immunohistochemistry 174

2. Analysis of Cell Culture Medium 176

2.1. Enzyme Linked Immunosorbent Assays 176

2.2. Uronic Acid Analysis 180

2.3. Iduronic Acid Analysis 181

2.4. Cellulose Acetate Electrophoresis 182

2.5. Thin Layer Chromatography 183

2.6. Gel Electrophoresis and Immunoblotting 184

3. Glycosaminoglycans and Proteoglycans of the Fetal Human TMJ Articular Disc 185

3.1. Hyaluronic Acid 185

3.2. Chondroitin Sulphate Proteoglycan 186

3.3. Dermatan Sulphate 188

3.4. Keratan Sulphate 188

4. Conclusion 190

5. Future Studies 192

5.1. Growth and Development Studies 192

5.1.1. Animal Studies 192

5.1.2. Human Studies 193

5.2. Studies of the TMJ Articular Disc in Dysfunction 194

5.2.1. Animal Studies 194

5.2.2. Human Studies 194

5.3. Applied Studies 195

5.3.1. Biochemical Content and Diagnostic Accuracy of MRI 195

1. Microscopy of the Fetal TMJ Articular Disc

1.1. Histology

The fetal human TMJ articular disc, at 22 to 25 weeks i.u., was macroscopically recognized as a biconcave structure of approximately 4-5 mm in length with a thin central region and anterior and posterior rims of no more than one millimeter in thickness. The general structural morphology of the disc is consistent with earlier reports in the literature (Van der Linden *et al.*, 1987; Wong *et al.*, 1985; Furstman, 1963). Furstman (1963) described the articular disc at fourteen weeks intra-uterine and later in prenatal development as having a thin central area that becomes wider as the peripheral regions of the disc are reached. In contrast with the reports of Van der Linden *et al.* (1987) and Wong *et al.* (1985), which indicated that the thin central area of the fetal human TMJ articular disc is devoid of blood vessels, capillaries containing red blood cells were observed in the inferior region of the intermediate band.

An immunohistochemical assay for Factor VIII-related antigen was performed but did not provide definitive evidence of capillaries. However, this technique may be of limited use in developing and poorly differentiated tissues due to the limited quantities of antigen present (Angervall *et al.*, 1986).

Staining of the disc with Haematoxylin and Eosin as well as Masson's trichrome revealed that the disc was highly cellular with a dense arrangement of collagen fibers. The composition of the disc is in agreement with earlier reports by: Van der Linden *et al.* (1987); Wong *et al.* (1985); Keith (1982); Youdelis (1966); Furstman (1963); Strauss

(1960) and Symons (1952). The density of cells appeared to be higher in the intermediate band in comparison to the anterior and posterior bands. Cell counts in the anterior, intermediate and posterior bands revealed a ratio of 1:1.5:1, respectively. A distinct layer of cells was observed on the inferior surface of the intermediate band. The significance of these observations is not established, although one may reason that there must be a physiologic purpose for the increase in cellularity in these areas. Since joint forces at this stage of development are likely very minimal, if at all existant, a possible explanation may be an increased production of PGs in these areas in preparation for the forces of loading. This would suggest a predominantly genetic determination of the load-bearing role of the TMJ articular.

A relatively acellular layer was observed between the inferior surface of the intermediate band and the remainder of the disc. The absence of cells in this area gave the impression of cellular invasion in this region. This may indicate that the external morphology of the disc is formed prior to its internal composition.

The Taenzer-Unna Orcein stain revealed elastic fibers in both the anterior and posterior bands of the disc. Although absence of elastic fibers in the articular disc at 13 to 17.5 weeks i.u. has been noted (Wong *et al.*, 1985), the presence of elastic fibers has been reported in the anterior and posterior bands of the disc at 21 weeks i.u. (Morimoto *et al.*, 1987).

Keith (1979) observed two types of elastic fibers in the fetal bovine temporomandibular joint disc; the first type was distributed throughout the articular disc and ran primarily parallel to the collagen fibers, the second type was coarser and

extended from the upper posterior aspect of the disc to the cranial base.

Frommer and Monroe (1966) reported elastic fibers only in the peripheral portions of the temporomandibular joint articular disc of the fetal mouse and suggest that the absence of elastin in the central portion of the disc indicates that this area may not be particularly stress bearing. Their contention is that tensile forces are countered by elastic fibers. Miles and Dawson (1962) suggested that elastic fibers confer a degree of resilience upon articular fibrous tissue which, in articular cartilage, is provided by a high chondroitin sulphate PG content. However, one may infer from this that fibrous tissue with a high CSPG content would not require elastic fibers to cope with forces of loading, as the collagen fibers would counter tensile forces while the proteoglycans would resist compressive forces.

Alcian Blue and Safranin-*O* were used to stain for HA and sulphated GAGs in the articular disc. Alcian Blue stained fairly regularly throughout the disc, while Safranin-*O* stained the thinner central portion of the disc more intensely. The pattern of intense staining seemed to correspond with the areas of increased cellularity. This may further support the idea that there is higher production of proteoglycans in these areas. This result is in agreement with the Alcian Blue studies of Kopp (1976) which demonstrated a higher concentration of sulphated GAGs in the central portion of the adult human TMJ disc. Nakano and Scott (1989) also reported more intense staining with Safranin-*O* in the central portion of the adult bovine TMJ disc. It is remarkable that the fetal human TMJ disc would exhibit staining patterns similar to that of mature TMJ discs. This strongly suggests that the TMJ disc at this stage of fetal development has

already been assigned a load-bearing role.

Following enzymatic treatment of the disc sections with *Streptomyces* hyaluronidase and chondroitinase ACII, spectrophotometric absorbance after staining with Alcian Blue was reduced by 7.4% and 55% respectively. However, chondroitinase ACII digests both CS and HA. Therefore if 7.4% were subtracted from 55%, it would appear that a 47.6% reduction in absorbance was due to removal of CS. The ratio of HA to CS would appear to be 1:3.5. Although other tissues such as human skin and human fetal condylar cartilage were used as comparisons, they did not prove to be useful as they exhibited irregularities and separations which produced inconsistent spectrophotometric readings.

Kopp (1976) attributed the differential pattern of staining for sulphated GAGs to the different loads distributed in various areas of the disc. His reasoning was that since sulphated GAGs comprise a significant portion (10%) of the dry weight of adult weight-bearing articular cartilage (Mc Devitt, 1973), their presence in the central area of the TMJ disc indicates that this area also plays a role in load-bearing.

Studies of pathologic TMJ articular discs removed from patients diagnosed as having reducible and non-reducible disc displacements indicate that significant amounts of sulphated GAGs may be detected in the posterior band and bilaminar zone of the articular disc (Blaustein and Scapino, 1986). These investigators suggest that this finding is indicative of the ability of these tissues to remodel to increase their compressive stiffness in order to cope with a load-bearing function. However, they also caution that this remodelling response may not always be manifest.

1.2. Immunohistochemistry

Immunologic staining of the articular disc sections with monoclonal antibodies CS56 (αCS), 6D6 (αDSPG II), 5D4 (αKS) and NDOG1 (αHA) demonstrated; heavy staining for CS, minimal staining for DSPG II, no apparent staining for KS and moderate staining for HA. CS and HA staining appeared to be uniform, while DSPG II was observed to be more intense around the edges of the intermediate band of the disc.

Enzymatic digestions were utilized to remove GAGs to determine the specificity of the antibodies. Following treatment with chondroitinase ACII, staining with CS56 was significantly reduced although not eliminated. This enzyme had no effect on the 6D6 staining of the disc sections, although it did reduce staining with NDOG1. However, the latter result was not particularly surprising as the manufacturer (Seikagaku® USA) indicates that this enzyme exhibits activity for both HA and CS. Although this cross reactivity with HA is not well documented, this study shows that cross reactivity with HA may exist. The enzyme *Streptomyces* hyaluronidase only slightly reduced the staining intensity of antibody NDOG1. These data suggest that this antibody is not specific for HA. A subsequent digestion with chondroitinase ACII further reduced staining intensity of NDOG1. It appears that this antibody demonstrates activity for antigens other than HA, one of which may possibly be CS.

Since Safranin-*O* staining demonstrated more intense staining in the central portion of the disc, one would expect a high concentration of CS in this area, however, antibody staining with CS56 appeared to be quite uniform throughout the disc. This anomaly was also reported in the bovine articular disc by Nakano and Scott (1989). It

is likely that variations in chemical composition of GAGs occur in the disc, such as hybridization between C_4S, C_6S and DS, variation in degree of sulphation and molecular weight polydispersity (Granström and Linde, 1973). In view of the finding that sulphated GAGs are more abundant in areas resisting compression (Kopp, 1976), this anomaly may be accounted for by the distribution of the greater sulphated populations of CS in the central portion of the disc and the less or non-sulphated populations of CS occurring in the periphery of the disc.

A significant proportion (12%) of CS in fetal sheep cartilage has been reported to be non-sulphated (Dziewiatkowski *et al.*, 1989). This proportion decreases to 1% postnatally. It would seem possible that a population of non-sulphated CS may exist in the fetal human articular disc.

2. Analysis of Cell Culture Medium

2.1. Enzyme Linked Immunosorbent Assays

ELISA inhibition assays for CSPG using antibody CS56 demonstrated relatively large amounts of CSPG in DEAE1, 5 and 6 while there was considerably less in DEAE2, 3 and 4.

The findings for DEAE1, 5 and 6 are consistent with reports in the literature suggesting that fetal cartilaginous tissues contain large amounts of CS. Dziewiatkowski *et al.* (1989) reported large amounts of CS in fetal sheep cartilage while Melching and Roughley (1989) indicated that the major aggregating proteoglycan of human fetal articular cartilage carries predominantly C_6S side chains.

DEAE4 was derived from unconditioned cell culture medium containing 2% FCS and was found to contain a small amount of CSPG. This indicates that a quantity of CSPG in DEAE1, 2, 5 and 6 may actually be from the serum and not actually produced by the cells.

CSPG content of DEAE2 and 3 was very minimal compared to the other DEAE pools of conditioned medium. DEAE2 and 3 pools were collected from the same population of fibroblasts at different times. As discussed below, the history, cell line and age of the cells may be responsible for the reduced CSPG content.

During the course of the experiments, some interesting characteristics of monoclonal antibody CS56 were revealed. Inhibition assays showed that this antibody exhibited strong antigenic binding activity to C_6S but only minimal binding to C_4S. As

expected, there was no apparent binding to either the DSPG II or HA standards. The C_4S standard (from whale cartilage) contains (according to Seikagaku®) C_4S and C_6S in a ratio of 80:20. Therefore, the minimal binding observed with the C_4S standard may actually be to C_6S in the standard. This suggests that the ELISAs using CS56 may only be assaying for C_6S in the DEAE pools. Furthermore the C_6S standard (from shark cartilage) contains C_4S and C_6S in a ratio of 10:90. Another finding was that there was only a slight difference in CS56 binding activity towards a C_6S standard compared to CS attached to a protein core (CSPG). The averaged (from two trials) IC_{50} value for the CSPG standard was only slightly higher than the averaged IC_{50} value for the C_6S standard. One would expect the IC_{50} value of CSPG to be much higher because of the attached protein core. A possible explanation, may be the impurity of the C_6S standard. Impurities (such as the aforementioned C_4S) would add to the dry weight of the standard, resulting in a higher IC_{50}.

Compared to the CSPG values, quantities of DSPG II were considerably less. Larger amounts were found in DEAE1, 2, 5, and 6 while smaller quantities were found in DEAE2 and 3 and untreated fetal calf serum.

Using the formula: **%IdU.A. = [PDS] x 0.5 x 0.292 x 0.8/ Total U.A.**, the percentage iduronic acid was calculated to be 29%, 25% and 24% for DEAE1, 5 and 6 respectively. The conversion factor of 0.5 is due to the composition of PDS, which is composed of 50% protein and 50% GAG. A DS standard was analyzed to determine its uronic acid content of 29.2%. Therefore, a conversion factor of 0.292 was used. Lastly, the proportion of iduronic acid of total DS uronic acid is approximately 80%

(Gibson and Pearson, 1982) providing the final conversion factor of 0.8.

Although the untreated fetal calf serum did not seem to contain much DSPG II, the value of DEAE4 (unconditioned cell culture medium containing 2% FCS) was approximately half of the amount in conditioned cell culture medium. This suggests that the cells may only be producing very small amounts of DSPG II. DEAE3 (conditioned medium containing 0% FCS) contained small amounts of DSPG II suggesting that the cells are in fact producing DSPG II.

Although this study suggests that there are small amounts of DSPG II (PDS) in the fetal human TMJ articular disc, the presence of DSPG II is not unexpected as this molecule is thought to play an important role in determining the sizes and tensile properties of collagen fibrils (Fleischmajer *et al.*, 1991; Scott, 1988; Poole *et al.*, 1986).

Lastly KS content of the conditioned medium was minimal. This result is consistent with studies of fetal tissues which indicate that there is very little or no KS at this stage of development. Salisbury and Watt (1988) were unable to detect KS in the human fetal notochord using monoclonal antibody 5D4. Very little, if any, KS was found in the flexor tendon of fetal calves, although increasing amounts were found in pressure bearing regions of tendons of young calves (Evanko and Vogel, 1990). Evidence suggests that KS may be associated with aging of tissues as it is detected in higher amounts in tissues such as maturing articular cartilage (Dziewiatkowski *et al.*, 1989; Webber *et al.*, 1987) and aging human skin (Willen *et al.*, 1991).

The relative variation of proteoglycan production by the cultured fibroblasts in this study may be due to a number of factors involving their history, age, and cell strain.

Firstly, the history of the cells may contribute to this difference as cells producing conditioned medium for DEAE1, 5 and 6 were early passages of cells that were at no time subject to the effects of freezing and storage in liquid nitrogen. DEAE2 and 3 were consecutive collections of conditioned medium from the same population of cells which were earlier frozen for storage. It was observed that freezing of the cells had a profound effect on their metabolism and ability to proliferate as it took as long as 21 days for the cells to reach confluency compared to cells which had not undergone freezing, which became confluent in 7 to 10 days. Furthermore, many cells did not survive this process, suggesting that a population of cells was selected which may be quite different than the undisturbed original population. Secondly, aging of the cells *in vitro* may have an effect on their metabolism and PG production. Conditioned medium for DEAE3 was collected in 6 to 7 days after medium was collected for DEAE2 from the same population of cells. Lastly, the cell lines were obtained from two fetuses at different ages of intra-uterine development which may also contribute variation.

A further explanation for the difference in proteoglycan values may be related to the original volume of conditioned medium available for the DEAE columns. Although all volumes were smaller than desired, the original volumes for DEAE5 and 6 were over twice that of the other DEAE pools. Following the PG extraction, concentration and recovery procedures, there were very limited quantities of PG to analyze (especially with the smaller DEAE1, 2, 3 and 4 pools). Subsequently, many analyses were performed on a half or otherwise reduced scale which may not be as accurate as the same full scale analysis.

DEAE3 contained a smaller amount of PG. This result was anticipated as production of PGs was expected to be significantly reduced in serum free medium. Malemud and Papay (1986) demonstrated a reduction in newly synthesized proteoglycan by rabbit chondrocytes under serum free cell culture conditions. Furthermore, they found a reduction in aggregation and in the hydrodynamic size of the larger proteoglycan monomers following even one day of exposure to serum free medium.

Although the presence of serum in the conditioned medium confounds the data because basal levels of serum PGs are not established, the absence of serum appears to have a major effect on PG production, size and aggregation. This is an inherent problem with the culture of mammalian cells and should always be kept in mind whenever comparisons are made with the *in vivo* situation.

2.2. Uronic Acid Analysis

Using the technique of Kosakai and Yosizawa (1978) uronic acid content was demonstrated to be greater in DEAE1, 5 and 6. Relatively smaller amounts were found in DEAE2, 3 and 4.

This result seems to generally support the ELISA inhibition assay data for CSPG as significantly larger quantities of CSPG were found in DEAE1, 5 and 6 compared to the other DEAE pools. However, the uronic acid values are very low in comparison to the ELISA values for CSPG content. A possible explanation for these data may be that the CSPG produced by the cells of fetal TMJ articular disc is more of a versican type where there are only approximately 15 side chains (Zimmerman and Ruohslohti, 1989)

compared to the aggrecan type with approximately 50 or more side chains (Poole, 1986). Furthermore, the ELISA values for CSPG may be over-estimated. The antibody CS56 exhibits cross-reactivity to heparan sulphate (Avnur and Geiger, 1984). However, the degree of overestimation is unknown because the levels of heparan sulphate were not established.

2.3. Iduronic Acid Analysis

Iduronic acid content was determined using the Di Ferrante method (Di Ferrante *et al.*, 1971) of dermatan sulphate measurement. Greater amounts of iduronic acid were found in DEAE1 and 2 while lesser amounts were found in DEAE3, 4 ,5 and 6.

These results are not consistent with either the ELISA results for DSPGII or the uronic acid values. It may be possible that this technique is not sensitive for small quantities such as the DEAE pools obtained from an original cell culture medium volume of 220 ml. DEAE5 and 6 were derived from larger initial volumes and produced more manageable amounts of sample. Another confounding variable may be the purity of the sample. Di Ferrante *et al.* (1971) caution that impurities of glycogen and glycoprotein interfere with the analysis. Using the values of DEAE5 and 6 the percentage of iduronic acid was calculated to be 25% and 41% of total uronic acid content. The ELISA results for DSPGII indicate that iduronic acid is approximately 24-29% of total uronic acid, which is in accordance with the value of 25% for DEAE5.

2.4. Cellulose Acetate Electrophoresis

In general, the electrophoretograms showed two peaks for DEAE1, 2, 4, 5 and 6. The first peak was in the vicinity of the HA standard suggesting that it was HA. This was subsequently confirmed with digestions with *Streptomyces* hyaluronidase, as this peak was not present in treated samples. The other peak was generally larger with a broad base extending across both CS and DS standards and its apex occurring in the area of the DS standard. Although it is reasonable to accept that this peak contains both CS and DS, it is difficult to comment on the exact composition of this peak. However, this second peak was noticeably diminished in DEAE5 and 6 following treatment with chondroitinase B, strongly suggesting that DS was present.

Granström and Linde (1973) used similar electrophoretic methods to determine the glycosaminoglycans of TMJ articular discs of the rat, rabbit, dog and monkey. Similarly, they observed two peaks on the electrophoretograms as well, with the initial one having the same electrophoretic mobility as HA and the second predominant peak having the same mobility as DS. These authors were unable to determine the composition of the latter peak.

DEAE3 (conditioned medium with 0% FCS) appeared as a broad band with no clear demarcations. There was only a very slight rise in the area of the HA standard and the majority of the band occurred at the same position as the CS and DS standards.

DEAE4 (unconditioned medium with 2% FCS) was found to contain a significant amount of HA. This finding was not surprising as fetal calf serum was anticipated to contain some HA. However, this result makes it difficult to determine the contribution

of HA from the serum in comparison to the quantity produced by the cells. There are currently limited studies on the HA content of fetal calf serum. However, Engström-Laurent *et al.* (1985) observed hyaluronic acid to be approximately 30 to 40 μg/l in blood plasma of healthy adult humans.

HA was calculated to be 33%, 30%, 33% and 32% of the total GAGs of DEAE1, 2, 5 and 6 respectively.

2.5. Thin Layer Chromatography

Although this technique was efficient in separating the disaccharide standards, it was less effective with the DEAE sample. Light color development appeared with the same mobility as the ΔDi-0S standard. However, from these results, one cannot be definite if this color is due to impurities, non-sulphated GAGs or if it is due to a "trail" of buffer. The analyzed DEAE samples were of relatively low purity compared to the standards. Sample sizes were too small to permit further purification.

It would not be unexpected to find non-sulphated GAGs such as HA disaccharides in the DEAE sample. As discussed earlier in the immunohistochemical results, it is possible that a small population of non-sulphated GAGs, such as non-sulphated CS exists in the fetal human TMJ articular disc. Both the immunohistochemical data and the cellulose acetate data indicate that there are significant quantities of HA in the fetal articular disc.

2.6. Gel Electrophoresis and Immunoblotting

The SDS-PAGE gels revealed that some of the proteoglycans in DEAE5 and 6 had a molecular size close to the CSPG I standard from adult bovine TMJ articular disc. Following digestion with chondroitinase ABC, the core of these proteoglycans were compared to molecular weight standards and estimated to be greater than 200 K.

Antibodies 6D6 (αDSPG II) and CS56 (αCS) were used to verify the presence of DSPG II and CS in the DEAE samples. The location of the antibody stains corresponded to the DSPG II and CSPG I standard respectively. Immunoblotting results are consistent with the ELISA data.

These findings are in accordance with studies which indicated that fetal cartilage proteoglycans are predominantly large PGs rich in CS (Mallinger and Stockinger, 1987). Buckwalter *et al.* (1985) compared the aggregating and non-aggregating cartilage proteoglycan monomers in calves with the corresponding ones in steers to find that the calf monomers were longer and less variable in length than those of the steer. Theocharis and Tsiganos (1985) reported similar findings in sheep nasal cartilage. They found larger aggregatable proteoglycans with higher chondroitin sulphate content in younger sheep.

Studies of aging and maturation of cartilage have shown that in older cartilage the PGs are smaller, have shorter side chains and contain more KS (Glant *et al.*, 1986; Roughley and White, 1980; Bayliss and Ali, 1978; Inerot *et al.*, 1978). Furthermore, these studies suggest that adult cartilage contains multiple polydisperse populations of PGs with more than one core protein.

3. Glycosaminoglycans and Proteoglycans of the Fetal Human TMJ Articular Disc

The relative proportions of GAGs in the fetal human TMJ articular disc appear to be: 32% HA; 42% CS; 26% DS with very minute quantities of KS.

3.1. Hyaluronic Acid

The immunohistochemical results indicated that there were significant quantities of HA present. The cellulose acetate experiments quantified the proportion of HA to be approximately 32%, although the basal level of HA in the serum was not known. This value supports the literature in that relatively large proportions of HA exist in proliferating tissues (Toole *et al.*, 1984). HA is thought to play an important role in cell proliferation, migration and invasion by producing and maintaining an open, loose extracellular matrix structure (Toole, 1981; Comper and Laurent, 1978).

However, the value of 32% HA is at best only a rough approximation, as HA production can vary widely as a function of tissue culture conditions (Huey *et al.*, 1990). Ascorbic acid in the serum is observed to have a general anabolic effect on cells in culture and enhances matrix assembly (Wright *et al.*, 1988). Huey *et al.* (1990) studied the effects of ascorbic acid, glucose, serum and culture medium on the incorporation of HA by human skin fibroblasts in culture. They found a variation of 20 to 30 μg of ascorbic acid/ml present in FCS (GIBCO®). The optimum level of ascorbic acid was found to be 25 μl/ml as greater levels inhibited HA incorporation. Increasing glucose

levels from 0.1 to 0.2 to 0.45% produced a decreasing trend in HA incorporation. They also compared fetal calf serum (FCS) with new-born calf serum (NBCS) and calf serum to find that FCS was more than twice as effective as new-born calf serum and six times as effective as calf serum in incorporating [^{3}H]glucosamine into HA. Lastly, different culture medium was found to produce variation in cell proliferation.

A serum factor that stimulates HA has been documented (Tomida *et al.*, 1975, 1977; Knudson *et al.*, 1984) and characterized (Decker *et al.*, 1989). These investigators suggest that this factor is essential for production and metabolism of HA by cells in culture.

3.2. Chondroitin Sulphate Proteoglycan

Glycosaminoglycans from CSPG account for approximately 42% of the total GAGs. The immunohistochemical results indicated large proportions of CS in the articular disc. The spectrophotometric readings of the Alcian Blue stained sections decreased by 47.6% following removal of CS with chondroitinase ACII. The value of 42% is obtained by subtracting both the HA (32%) and DS (26%) percentages from 100% (total GAGs).

This result is in general agreement with reports in the literature of high amounts of CS in fetal tissues such as fetal sheep cartilage (Dziewiatkowski *et al.*, 1989; Theocharis and Tsiganos, 1985) and fetal human articular cartilage (Melching and Roughley, 1989; Mallinger and Stockinger, 1987).

The histochemical and thin layer chromatography results suggest that there may

be a population of non-sulphated CS in the disc. A significant proportions of non-sulphated CS has been reported in fetal sheep cartilage (Dziewiatkowski *et al.*, 1989). It would seem possible that a non-sulphated population of CS exists in the fetal articular disc.

The SDS-PAGE results indicate that the chondroitinase ABC produced core of CSPG from the DEAE pools is very large and estimated to be in excess of 200 kDa. This description is supportive of the reports of fetal human articular cartilage that suggest the predominant PG is large (Mallinger and Stockinger, 1987).

Melching and Roughley (1989) reported that the major aggregating PG of human fetal articular cartilage carries predominantly C_6S chains. Results with monoclonal antibody CS56 seem to indicate that the major portion of CS is C_6S in the fetal TMJ articular disc. Since the antibody CS56 demonstrated very weak activity for C_4S, no inferences regarding the proportion of C_4S can be made. Dziewiatkowski *et al.* (1989) reported an increase in the ratio of C_4S to C_6S post natally to 1:7 in the scapular cartilage of sheep. Furthermore, they also reported a reduction in the size of the PG monomers after one year of age. Vasan *et al.* (1991) observed a shift in PG molecular size from larger to smaller moieties in the intervertebral discs of older dogs. They suggest that this feature may be associated with an age-related inability of the intervertebral disc to tolerate stress.

In summary, the major PG of the fetal human TMJ articular disc appears to be a CSPG with predominantly C_6S GAG side chains and a core of larger than 200 kDa. This suggests that the disc at this stage of development is more cartilage-like than

fibrous-like.

3.3. Dermatan Sulphate

Based on the ELISA results and the iduronic acid analysis, DS was estimated to comprise 26% of the total GAGs. The presence of DSPG II in the sample was confirmed by SDS-PAGE electrophoresis and immunoblotting on nitrocellulose paper using a DSPG II standard.

The proteoglycan DSPG II (PDS) has been reported in many fetal tissues. Melching and Roughley (1989) found that the major DSPG to be synthesized in the fetal human articular cartilage is DSPG I although they did find reasonable amounts of DSPG II. Evanko and Vogel (1990) reported both DSPG I and DSPG II in the fetal bovine flexor tendon. Poole *et al.*(1986) detected DSPG II only in the superficial layers of adult articular cartilage, while it was found to be distributed relatively evenly in fetal articular cartilages.

DSPG II may play an important role in determining the sizes and tensile properties of collagen fibrils (Poole *et al.*, 1986: Scott *et al.*, 1981). The presence of DSPG II in the fetal TMJ articular disc would attest to its highly collagenous structure.

DSPG II may also play a role in aging of tissues as this PG is significantly reduced in the articular cartilage of older subjects (Vilim and Krajickova, 1990).

3.4. Keratan Sulphate

Although KS was not detected by immunohistochemical staining, it was measured

with the more sensitive ELISA inhibition assay. A very small quantity of KS was detected.

Studies seem to indicate that there are very minute or undetectable amounts of KS in fetal tissues. Salisbury and Watt (1988) reported no evidence of KS in the human notochord, while Evanko and Vogel (1990) reported minimal amounts of KS in the flexor tendon of fetal calves. Although KS is not prominent in fetal tissues, genetic studies indicate that fetal bovine cartilage PG contains the KS attachment domain, although it lacks the KS side chains (Antonsson *et al.*, 1989). KS appears to be associated with aging of tissues, as it is found in larger amounts in maturing tissues (Dziewiatkowski *et al.*, 1989; Webber *et al.*, 1987). Stanescu *et al.* (1988) observed fragments of large monomers containing KS in older human articular cartilage.

4. Conclusion

The fetal human TMJ articular disc at 22 and 25 weeks i.u. was found to be a highly cellular biconcave structure with a greater concentration of cells in the intermediate band. A dense arrangement of collagen fibers was found throughout the disc. Furthermore, a relatively acellular layer was observed immediately above the inferior surface of the intermediate band. Capillaries containing red blood cells were found along the inferior surface of the intermediate band of the disc.

The disc was enriched with PGs and GAGs which included HA, CSPG and DSPG II. Relative percentages of total GAGs appeared to be 32% HA, 42% CS, 26% DS and very little KS. A large proportion of HA was not surprising as HA is abundant in proliferating tissues and is associated with cell proliferation and organization (Toole, 1981; Comper and Laurent, 1978). The large proportion of CS is consistent with reports in the literature of high amounts of CS in fetal cartilage(Dziewiatkowski *et al.*, 1989; Theocharis and Tsiganos, 1985) and fetal articular cartilage (Melching and Roughley, 1989; Mallinger and Stockinger, 1987). The main type of CS appeared to be C_6S which is also the major GAG of human fetal articular cartilage (Melching and Roughley, 1989). The CSPG core was estimated to be larger than 200 kDa. Large aggregatable PGs may function in proliferating tissues to maintain large hydrodynamic volumes which assist in maintaining a loose open matrix for cell proliferation and migration. The presence of DSPG II may be associated with the dense arrangement of collagen fibers in the disc. DSPG II is thought to play a role in determining the sizes and tensile

properties of collagen fibrils (Poole *et al.*, 1986; Scott *et al.*, 1981). Finally, KS was detected in very small amounts, which is typical of many other fetal tissues.

5. Future Studies

5.1. Growth and Development Studies

At present, there are very limited studies concerning the biochemical changes that occur in the TMJ articular disc during normal growth and development. Knowledge of the biochemical and cellular changes that occur in the disc in the normal situation is essential in providing meaningful insights into the accompanying biochemical and cellular changes of TMJ dysfunction.

5.1.1. Animal Studies

Growth and development of the TMJ articular disc may be studied using an animal model. The advantages of animal studies are many; animals may be raised in controlled environments, their life spans are relatively shorter and the investigator may select the precise age for study. A reasonable choice of animal for this type of study would be a guinea pig or rodent such as a mouse or a rat.

Methods may be similar to this study, with one articular disc used for microscopic methods which include histologic and immunohistochemical analysis and the other disc reserved for culture of cells. Following cell culture, the conditioned medium may be analyzed with similar methods. Ages of study could be pre-natal, at birth, during growth, and adult.

In conjunction with these methods, the use of radioisotopes may provide more precise values for total sulphated GAGs (^{35}S) and HA content of the conditioned medium

(^{125}I).

The purpose of such a study would be to survey the biochemical changes that occur in the articular disc with normal growth and development. If a trend could be established, this knowledge may be extrapolated to human studies.

Pig or bovine material may also be considered for this type of study. Material may be obtained at an abattoir. However, the samples obtained would likely be only of fetal and adult ages. An advantage of this material would be the amount available as the discs are considerably larger than that of laboratory animals.

5.1.2. Human Studies

TMJ articular discs obtained at autopsy may be examined microscopically with histologic and immunohistochemical methods. However, cell culture may not always be feasible, as fibroblasts only remain viable for approximately 5 to 7 days post-mortem. Range of ages of study may be pre-natal, at birth, infant, child, adolescent, adult, and senior. Selection would be based on the absence of signs and symptoms of TMJ dysfunction. Herein lies one of the major obstacles to this type of study as there is no assurance that all samples are without dysfunction and are representative of the normal situation. A further problem may the availability of human material for this type of study.

The use of cDNA probes to DSPG II and CSPG (versican) may add another perspective to studies of the articular disc. An advantage of *in situ* studies is that they are able to gauge cellular production and activity with much more accuracy than cell

culture studies. Furthermore, cellular activity in different regions of the disc may be observed.

5.2. Studies of the TMJ Articular Disc in Dysfunction

5.2.1. Animal Studies

Hypotheses regarding the specific role of GAGs and PGs may be tested with animal models. Agents may be used to block or otherwise alter a PG function to study the sequelae. For example, chlorcyclizine, an antihistaminic benzhydrylpiperazine compound, enhances the degradation of HA and chondroitin sulphate with little effect on their synthesis (Wilk *et al.*, 1982).

5.2.2. Human Studies

TMJ articular discs that are distorted or perforated may be obtained at the time of surgery or autopsy. Histologic and immunohistochemical methods may be utilized to study the distribution of PGs in the disc in these situations. The disc may be divided into sections and each section used as an explant for culture of cells. It would be interesting to determine if there is a variation in PG production in the different areas of the disc. Furthermore, in cases where function has been on the bilaminar zone for some time, the cells in this area may be cultured to determine the type and quantities of PGs that they are producing.

The techniques of *in situ* hybridization may be applied to pathologic discs as well to provide insights into the cellular activity of the disc in conditions such as disc derangement.

5.3. Applied Studies

5.3.1. Biochemical Content and Diagnostic Accuracy of MRI

Knowledge of the biochemical content of the human TMJ articular disc may be applied to the diagnosis of TMJ dysfunction. However, this relationship has not been established. A relatively recent advance in the evaluation of internal derangements of the TMJ is magnetic resonance imaging (MRI). This technique is favourable because of its ability to image soft tissue and its non-invasiveness. Unfortunately, at this time access and cost are limiting factors in many areas.

The correlation between the biochemical content of the TMJ disc and its appearance on an MRI image may be studied with an *in vitro* method. Theories concerning the biochemical content of the TMJ articular disc in the "normal" and "abnormal" situation could be tested by fabricating gels containing PGs and GAGs and imaging them with MRI. The PG and GAG content of the gels may be altered to determine if the accompanying change in hydrodynamic volume is detectable on the MRI image. These results may then be compared to images of the TMJ articular disc of patients. If a relationship between biochemical content of the TMJ disc and the MRI image were established, MRI may become a more useful tool in our understanding of the TMJ.

CHAPTER SIX

BIBLIOGRAPHY

BIBLIOGRAPHY*

Abdel-Fattah RA. Optimum Temporomandibular Joint (TMJ) Condylar Position. *Todays-FDA* 1989;1(3):1C-3C.

Adams ME, Muir H. The Glycosaminoglycans of Canine Menisci. *Biochem J* 1981;197:385-9.

Alberts B, Bray D, Lewis J, Raff M, Roberts K, Watson JD. *Molecular Biology of the Cell.* 2nd Ed., New York., Garland Publishing Inc., 1989.

Alwan WH, Carter SD, Bennett D, May SA, Edwards GB. Cartilage Breakdown in Equine Osteoarthritis: Measurement of Keratan Sulphate by an ELISA System. *Res Veterin Sci* 1990;49:56-60.

Angervall L, Kindblom LG, Rydholm A, Stener B. The Diagnosis and Prognosis of Soft Tissue Tumours. *Seminars in Diagnostic Pathology* 1986;3:240.

Antonopoulos CA, Axelsson I, ard D, Gardell S. Extraction and Purification of Proteoglycans from Various Types of Connective Tissue. *Biochim Biophys Acta* 1974;338:108-19.

Antonsson P, Heinegärd D, Oldberg A. The Keratan Sulfate-Enriched Region of Bovine Cartilage Proteoglycan Consists of a Consecutively Repeated Hexapeptide Motif. *J Biol Chem* 1989;264(27):16170-3.

Aplin JD, Hughes RC. Complex Carbohydrates of the Extracellular Matrix Structures, Interactions and Biological Roles. *Biochem Biophys Acta* 1982;694:375-418.

Appleton J. The Ultrastructure of the Articular Tissue of the Mandibular Condyle in the Rat. *Arch Oral Biol* 1975;20:823.

Avery JK. *Oral Development and Histology.* Avery JK. (Ed.)., Baltimore MD., Williams and Wilkins., 1987.

Avnur Z, Geiger B. Immunocytochemical Localization of Native Chondroitin-Sulfate in Tissues and Cultured Cells Using Specific Monoclonal Antibody. *Cell* 1984;38:811-22.

---

*References are in Accordance with Index Medicus (1992)

Avnur Z, Geiger B. Spatial Interrelationships between Proteoglycans and Extracellular Matrix Proteins in Cell Cultures. *Exp Cell Res* 1985;158:321-32.

Baker JH, Caterson B. The Isolation and Characterization of the Link Proteins from Proteoglycan Aggregates of Bovine Nasal Cartilage. *J Biol Chem* 1979;254:2387-93.

Balazs EA. The Physical Properties of Synovial Fluid and the Special Role of Hyaluronic Acid. In: *Disorders of the Knee*. Vol.4., Helfet AJ. (Ed.)., Philadelphia., Lippincott., 1982.

Balazs EA. Structure and Metabolism of Connective Tissue Under Physiological and Pathological Conditions. In: *Arthritis and Osteoarthritis*. Ruttner J, *et al*. (Eds.)., Wein., Verlag Hans Huber., 1971.

Balazs EA. Some Aspects of the Aging and Radiation Sensitivity of the Extracellular Matrix with Special Regard to Hyaluronic Acid in Synovial Fluid and Vitreous. In: *Thule International Symposium: Aging of Connective and Skeletal Tissue*. Engel A, Loarsson T. (Eds.)., Stockholm., Nordiska Bokhandelns Forlag., 1969.

Balazs EA. Viscoelastic Properties of Hyaluronic Acid and Biological Lubrication. *Univ Mich Ctr J* (Special Issue) 1968;December:255.

Balazs EA, Gibbs DA. The Rheological Properties and Biological Function of Hyaluronic Acid. In: *Chemistry and Molecular Biology of the Intercellular Matrix*. Balazs EA. (Ed.)., London., Academic Press.,1970.

Balazs EA, Watson D, Duff IF, *et al*. Hyaluronic Acid in Synovial Fluid: I. Molecular Parameters of Hyaluronic Acid in Normal and Arthritic Human Fluids. *Arthritis Rheum* 1967;10:357-76.

Balazs EA, Bloom GD, Swann DA. Fine Structure and Glycosaminoglycan Content of the Surface Layer of Articular Cartilage. *Fed Proc* 1966;25:1813-6.

Baldioceda F, Pullinger AG, Bibb CA. Relationship of Condylar Bone Profiles and Dental Factors to Articular Soft-tissue Thickness. *J Craniomandib Disord Facial Oral Pain* 1990;4(2):71-9.

Bancroft JD, Stevens A. *Theory and Practice of Histological Techniques*. 3rd Ed., Edinburgh., Churchill Livingstone., 1990.

Barbenel JC. The Mechanics of the Temporomandibular Joint - A Theoretical and Electromyographical Study. *J Oral Rehab* 1974;1:19-27.

Barnett CH, Cochrane W, Palfrey AJ. Age Changes in Articular Cartilages in Rabbits. *Ann Rheumatic Diseases* 1963;22:389.

Barnett CH, Davies DW, MacConaill MC. *Synovial Joints.* London., Longmans., 1961.

Barry F. Genetic and Structural Organization of Proteoglycans *Biochem Soc Trans* 1990;18:197-8.

Bartold PM. Proteoglycans of the Periodontium: Structure, Role and Function. *J Periodont Res* 1987;22(6):431-44.

Bartold PM. Interleukin-1 Stimulates Proteoglycan and Hyaluronic Acid Production by Human Gingival Fibroblasts *in Vitro. Austral Dent J* 1988a;33(6):467-75.

Bartold PM. The Effect of Interleukin-1ß on Hyaluronic Acid Synthesized by Adult Human Gingival Fibroblasts *in Vitro. J Periodont Res* 1988b;23:139-47.

Bartold PM, Page RC. The Effect of Chronic Inflammation on Gingival Connective Tissue Proteoglycans and Hyaluronic Acid. *J Oral Path* 1986a;15:367-74.

Bartold PM, Page RC. Hyaluronic Acid Synthesized by Fibroblasts Cultured From Normal and Chronically Inflammed Human Gingivae. *Collagen Rel Res* 1986b;6:365-78.

Baume, LJ. Ontogenesis of the Human Temporomandibular Joint 1. Development of the Condyles. *J Dent Res* 1962;41(6):1327-39.

Baume LJ. Ontogenesis of the Human Temporomandibular Joint 2. Development of the Temporal Components. *J Dent Res* 1970;49(4): 864-75.

Bayliss MT. Proteoglycans: Structure and Molecular Organization in Cartilage. In: *Connective Tissue Matrix.* Hukins DWL. (Ed.)., London., McMillan., 1984;55.

Bayliss MT. Proteoglycan Structure and Metabolism During Maturation and Aging of Human Articular Cartilage. *Biochem Soc Trans* 1990;18(5):799-802.

Bayliss MT, Ali SY. Age-Related Changes in the Composition and Structure of Human Articular Cartilage Proteoglycans. *Biochem J* 1978;176:683.

Bayliss MT, Johnstone B, O'Brien JP. 1988 Volvo Award in Basic Science. Proteoglycan Synthesis in the Human Intervertebral Disc. Variation with Age, Region and Pathology. *Spine* 1988;13(9):972-81.

Bayliss MT, Venn M, Maroudas A, Ali SY. Structure of Proteoglycans from Different Layers of Human Articular Cartilage. *Biochem J* 1983;209(6):387-400.

Bean LR, Thomas CA. Significance of Condylar Positions in Patients with Temporomandibular Disorders. *J Am Dent Assoc* 1987;114(1):76-7.

Beaty NB, Mello RJ. Extracellular Mammalian Polysacharides: Glycosaminoglycans and Proteoglycans. *J Chromatography* 1987;418:187-222.

Bell WE. *Temporomandibular Disorders: Classification, Diagnosis, Management.* 2nd Ed., Chicago., Year Book Medical Publishers Inc., 1986.

Bell WE. *Temporomandibular Disorders: Classification, Diagnosis, Management.* 3rd Ed., Chicago., Year Book Medical Publishers Inc., 1990.

Bellinger DH. Present Status of Arthrosis of the Temporomandibular Joint. *J Oral Surg* 1948;6;9-16.

Bermejo-Fenoll A, Puchades-Orts A, Sanchez del Campo F, Panchon-Ruiz A, Herrera-Lara M. Morphology of the Meniscotemporal Part of the Temporomandibular Joint and its Biomechanical Implications. *Acta Anat* 1987;129:220-6.

Bertrand J, Delpech B, Maingonnat C, Bertrand P, Picard J. The Quantification of Pleural Hyaluronic Acid by an Immunoenzymoassay. *Ann Clin Biochem* 1990;27:167-8.

Bewyer DC. Biochemical and Physiologic Processes Leading to Internal Derangement with Adhesion. *J Craniomandib Disord Facial Oral Pain* 1989;3(1):44-9.

Bianco P, Fisher LW, Young MF, Termine JD, Robey PG. Expression and Localization of the Two Small Proteoglycans Biglycan and Decorin in Developing Human Skeletal and Non-skeletal Tissues. *J Histochem Cytochem* 1990;38(11):1549-63.

Bjelle AO. Variation in Content and Compositions of Glycosaminoglycans within the Articular Cartilage of the Lower Femoral Epiphysis of an Adult. *Scand J Rheumatol* 1974;3:81-8.

Bhaskar SN. *Orban's Oral Histology and Embryology.* 8th Ed. Bhaskar SN. (Ed.)., St. Louis., Mosby-Year Book Inc., 1976.

Bhaskar SN. *Orban's Oral Histology and Embryology.* 11th Ed. Bhaskar SN. (Ed.)., St. Louis., Mosby-Year Book Inc., 1991.

Blackwood HJJ. Adaptive Changes in the Mandibular Joints with Function. *Dent Clin North Am* 1966;10:559-66.

Blaustein DI, Scapino RP. Remodeling of the Temporomandibular Joint Disk and Posterior Attachment in Disk Displacement Specimens in Relation to Glycosaminoglycan Content. *Plastic Reconstuct Surg* 1986;78(6):756-64.

Blaustein DI, Heffez LB. *Arthroscopic Atlas of the Temporomandibular Joint.* Philadelphia., Lea and Febiger., 1990.

Bollen AM, Carslon DS, Dziewiatkowski DD. Aggregation of Proteoglycans in Cephalic, Epiphyseal and Articular Cartilages in Growing Sheep. *Connect Tiss Res* 1990;24(2):121-7.

Bouvier M. Effects of Age on the Ability of the Rat Temporomandibular Joint to Respond to Changing Functional Demands. *J Dent Res* 1988;67(9):1206-12.

Brand JW, Whinery JG, Anderson QN, Keenan KM. Condylar Position as a Predictor of Temporomandibular Joint Internal Derangement. *Oral Surg Oral Med Oral Pathol* 1989a;67(4):469-76.

Brand JW, Whinery JG, Anderson QN, Keenan KM. The Effects of Temporomandibular Joint Internal Derangement and Degenerative Joint Disease on Tomographic and Arthrotomographic Images. *Oral Surg Oral Med Oral Pathol* 1989b;67(2):220-3.

Brandt KD, Muir H. Heterogeneity of Protein-Polysaccharides of Porcine Articular Cartilage. The Chondroitin Sulphate Proteins Associated with Collagen. *Biochem J* 1971;123:747-55.

Breuer B, Quentin E, Cully Z, Gotte M, Kresse H. A Novel Large Dermatan Sulfate Proteoglycan from Human Fibroblasts. *J Biol Chem* 1991;266(20):13224-32.

Brinkley LL, Morris-Wiman J. Computer-Assisted Analysis of Hyaluronate Distribution During Morphogenesis of the Mouse Secondary Palate. *Development* 1987a;100:629-35.

Brinkley LL, Morris-Wiman J. Effects of Chlorcyclizine-Induced Glycosaminoglycan Alterations on Patterns of Hyaluronate Distribution During Morphogenesis off the Mouse Secondary Palate. *Development* 1987b;100:637-40.

Bronson RE, Argenta JP, Siebert EP, Bertolami CN. Distinct Fibroblastic

Subpopulations in Skin and Oral Mucosa Demonstrated by Differences in Glycosaminoglycan Content. *In Vitro Cell Dev Biol* 1988;24(11):1121-6.

Buchner R. Induce Growth of the Mandibular Condyle in the Rat. *J Oral Rehab* 1982;9:7.

Buchwalter JA, Kuettner KE, Thonar EJ. Age-Related Changes in Articular Cartilage Proteoglycans: Electron Microscope Studies. *J Orthop Res* 1985;3(3):251-7.

Buchwalter JA, Smith KC, Kazarien LE, Rosenberg LC, Ungar R. Articular Cartilage and Intervertebral Disc Proteoglycans Differ in Structure: An Electron Microscopic Study. *J Orthop Res* 1989;7(1):146-51.

Cabrini R, Erasquin J. The Articulation of the Temporomandibular Joint of the Rat. *Rev Odontol de Buenos Aires* 1941;7:1.

Caltabiano M, Caltabiano C, Martinez G, Leonardi R. Histology of the Cartilage of the Human Fetal Condyle. *Mondo-Ortod* 1990;15(4): 439-42.

Carey DJ. Biological Functions of Proteoglycans: Use of Specific Inhibitors of Proteoglycan Synthesis. *Mol Cell Biochem* 1991;104(1-2):21-8.

Carlson DS, McNamara JA, Ribbens KA. *Developmental Aspects of Temporomandibular Joint Disorders.* Monograph Number 16., Craniofacial Growth Series. Center for Growth and Development, University of Michigan, Ann Arbor, Michigan. 1985.

Carlsson GE, Öberg T. Remodeling of the Temporomandibular Joints. In: *Oral Sciences Reviews 6.* Melcher AH, Zarb GA. (Eds.)., Copenhagen., Munksgaard., 1975:53-86.

Carney SL, Muir H. The Structure and Function of Cartilage Proteoglycans. *Phsiol Rev* 1988;68(3):858-910.

Carson D, Fox R. Structure and Function of Synoviocytes. In: *Arthritis and Allied Conditions.* 10th Ed. McCarty D.(Ed)., Philadelphia., Lea and Febiger., 1985.

Carvalho RS. *The Synthesis of Collagen and Glycosaminoglycans in Vitro in Rat and Mouse Temporomandibular Disc.* Masters Thesis., University of Manitoba., 1990.

Cassaro A, Pitini A. Influence of Condylar Position and Translation Movement in the Development of Temporomandibular Dysfunction. *Stomatol Mediterr* 1988;8(4):395-

403.

Caterson B, Christner JE, Baker JR. Identification of a Monoclonal Antibody that Specifically Recognizes Corneal and Skeletal Keratan Sulfate. *J Biol Chem* 1983;258:8848-54.

Charnley J. The Lubrication of Animal Joints. In: *The Proceedings of a Symposium of Biomechanics*. Institution of Mechanical Engineers., London., 1959.

Choi HU, Johnston TL, Pal S, Tang LH, Rosenberg L, Neame PJ. Characterization of the Dermatan Sulphate Proteoglycans, DS-PGI and DS-PGII, from Bovine Articular Cartilage and Skin Isolated by Octyl-Sepharose Chromatography. *J Biol Chem* 1989;264(5):2876-84.

Chopra RK, Pearson CH, Pringle GA, Fackre DS, Scott PG. Dermatan Sulphate is Located on Serine-4 of Bovine Skin Proteodermatan Sulphate. *Biochem J* 1985;232(1):277-9.

Christiansen EL, Thompson JR. *Temporomandibular Joint Imaging*. St. Louis., C.V. Mosby., 1990.

Christiansen EL, Thompson JR, Zimmerman G, Roberts D, Hasso AN, Hinshaw DB Jr, Kopp S. Computed Tomography of Condylar and Articular Disk Positions within the Temporomandibular Joint. *Oral Surg Oral Med Oral Pathol* 1987;64(6):757-67.

Christner JE, Brown ML, Dziewiatkowski DD. Interactions of Cartilage Proteoglycan with Hyaluronate. *J Biol Chem* 1979;254:4624-30.

Christner JE, Caterson B, Baker JR. Immunological Determinants of Proteoglycans. Antibodies Against the Unsaturated Oligosaccharide Products of Chondroitinase ABC-Digested Cartilage Proteoglycans. *J Biol Chem* 1980;255(15):7102-5.

Clark GT, Solberg WK. *Perspectives in Temporomandibular Disorders*. Chicago., Quintessence Publishing Co., Inc., 1987.

Clausen J. *Immunochemical Techniques for the Identification and Estimation of Macromolecules*. 3rd Ed., New York., Elsevier., 1988.

Coleman RD. Temporomandibular Joint: Relation of the Retrodiskal Zone to Meckel's Cartilage and Lateral Pterygoid Muscle. *J Dent Res* 1970;49(3):626-30.

Coleman RD, Weisengreen HH. Degenerative Changes in the Articular Disc in Later

Maturity. *J Dent Res* 1955 (abstract):65.

Comper WD, Laurent TC. Physiological Function of Connective Tissue Polysaccharides. *Physiological Reviews* 1978;58(1):255-315.

Copray JCVM, Jansen HWB, Duterloo HS. The Role of Biomechanical Factors in Mandibular Condylar Growth and Remodelling *in Vitro*. *In* Carlson DS, McNamara JA, Ribbens KA (Eds.). *Developmental Aspects of Temporomandibular Joint Disorders*. Monograph #16. Craniofacial Growth Series. Center for Human Growth and Development, University of Michigan, Ann Arbor, Michigan, 1985:235-69.

Copray JCVM, Dibbets JMH, Kantomaa T. The Role of Condylar Cartilage in the Development of the Tempromandibular Joint. *Angle Orthod* 1988(Oct):369-80.

Cunat JJ, Bhaskar SN, Weinmann JP. Development of the Squamo-Mandibular Articulation in the Rat. *J Dent Res* 1956;35:533.

Cutler BS. Dentofacial Changes Produced by a Modified Milwaukee Brace in *Macaca mulatta*: A Roentgenographic and Histologic Study. *Master's Thesis*, University of Washignton, Seattle, 1968.

Dahlberg AE, Dingman CW, Peacock AC. Electrophoretic Characterization of Bacterial Polyribosomes in Agarose-Acrylamide Gels. *J Mol Biol* 1969;41:139-47.

Damle SP, Coster L, Gregory JD. Proteoglycans Isolated from Pig Skin. *J Biol Chem* 1982;257:5523-8.

Darnell J, Lodish H, Baltimore D. *Molecular Cell Biology*. 2nd Ed., New York., W.H. Freeman and Company., 1990.

Day A, McQuillan CI, Termine JD, Young MR. Molecular Cloning and Sequence Analysis of the cDNA for Small Proteoglycan II of Bovine Bone. *Biochem J* 1987;248 (3):801-5.

Dayer E, Mathai L, Glant TT, Mikecz K, Poole AR. Cartilage Proteoglycan-Induced Arthritis in BALB/c Mice. *Arthritis-Rheum* 1990;33(9):1394-405.

de Bont L, Liem R, Boering G. Ultrastructure of the Articular Cartilage of the Mandibular Condyle: Aging and Degeneration. *Oral Surg Oral Med Oral Path*. 1985;60:631-41.

de Bont L, Liem R, Havinga P, Boering G. Fibrous Component of the TMJ Disc. In:

Temporomandibular Joint, Articular Cartilage Structure and Function. Druk, Van Denderen,eds. 1985.

deBont L, Liem R, Havinga P, Boering G. Spatial Arrangement of Collagen Fibrils in the Articular Cartilage of the Mandibular Condyle: A Light Microscopic and Scanning Electron Microscopic Study. In: *Temporomandibular Joint, Articular Cartilage Structure and Function.* Druk, Van Denderen,eds. 1985.

Delpech B, Bertrand P, Maingonnat C. Immunoenzymoassay of the Hyaluronic Acid-Hyaluronectin Interaction: Application to the Detection of Hyaluronic Acid in Serum of Normal Subjects and Cancer Patients. *Analyt Biochem* 1985;149:555-65.

Di Ferrante N. Turbidimetric Measurement of Acid Mucopolysaccarides and Hyaluronidase Activity. *J Biol Chem* 1956;220:303-6.

Di Ferrante N, Donnelly PV, Bergland RK. Colorimetric Measurement of Dermatan Sulphate. *Biochem J* 1971;124:549-53.

Dixon AD. Structure and Functional Significance of the Intra-articular Disc of the Human Temporomandibular Joint. *Oral Surg* 1962;15;48-61.

Dolwick MF. Diagnosis and Etiology. In: *Internal Derangements of the Temporomandibular Joint.* Helms CA, Katzberg RW, Dolwick MF. (Eds.)., San Fransisco., University of California Press., 1983.

Drachman DB, Sokoloff L. The Role of Movement in Embryonic Joint Development. *Dev Biol* 1966;14:401-20.

Dreessen D, Halata Z. Age-Related Osteo-Arthritic Degeneration of the Temporomandibular Joint in the Mouse. *Acta Anat Basel* 1990;139(1):91-6.

Drury RAB, Wallington EA. *Carleton's Histological Technique.* New York., Oxford University Press.

Dudhia J, Fosang AJ, Hardingham TE. Domain Structure and Sequence Similarities in Cartilage Proteoglycan *Biochem Soc Trans* 1990;18:198-200.

Dunphy WG, Brands R, Rothman JE. Attachment of Terminal *N*-Acetylglucosamine to Asparagine-Linked Oligosaccharides Occurs in Central Cisternae of the Golgi Stack. *Cell* 1985;40(2):463-72.

Dziewiatkowski DD, LaValley J, Beaudoin AG. Age Related Changes in the

Composition of Proteoglycans in Sheep Cartilages. *Connect Tiss Res* 1989;19(2-4):103-20.

Engel J. Domains in Proteins and Proteoglycans of the Extracellular Matrix with Functions in Assembly and Cellular Activites. *Int J Biol Macromol* 1991;13(3):147-51.

Engstrom-Laurent A, Laurent UGB, Lilja K, Laurent T. Concentration of Sodium Hyaluronate in Sodium. *Scand J Clin Lab Invest* 1985;45:497-504.

Evanko SP, Vogel KG. Ultrastructure and Proteoglycan Composition in the Developing Fibrocartilaginous Region of Bovine Tendon. *Matrix* 1990;10(6):420-36.

Evered D, Whelan J. *Function of the Proteoglycans.* Ciba Foundation Synposium., New York., Wiley., 1986.

Faltz LL, Caputo CB, Kimura JH, Schrode J, Hascall VC. Structure of the Complex Between Hyaluronic Acid, the Hyaluronic Acid Binding Region, and the Link Protein of Proteoglycan Aggregates from the Swarm Rat Chondrosarcoma. *J Biol Chem* 1979a;254:1381-7.

Faltz LL, Reddi AH, Hascall GK, Martin D, Pita JC, Hascall VC. Characteristics of Proteoglycans Extracted from the Swarm Rat Chondrosarcoma with Associative Solvents. *J Biol Chem* 1979b;254:1375-80.

Farndale RW, Buttle DJ, Barrett AJ. Improved Quantitation and Discrimination of Sulphated Glycosaminoglycans by Use of Dimethylene Blue. *Biochimica et Biophysica Acta* 1986;883:173-7.

Farndale RW, Sayers CA, Barrett AJ. A Direct Spectrophotometric Microassay for Sulfated Glycosaminoglycans in Cartilage Cultures. *Conn Tiss Res* 1982;9:247-8.

Farrar W. Diagnosis and Treatment of Anterior Dislocation of the Articular Disc. *NY Dent J* 1971;41(10):348-51.

Farrar WB, McCarty WL. The TMJ Dilemma. *J Ala Dent Assoc.* 1979a;63:19-26.

Farrar WB, McCarty WL. *A Clinical Outline of Temporomandibular Joint Diagnosis and Treatment. Normandie Study Group for TMJ Dysfunction.* Montgomery., Normandie Publications., 1979b.

Fellini SA, Hascall VC, Kimura JH. Localization of Proteoglycan Core Protein in Subcellular Fractions Isolated from Rat Chondrosarcoma Chondrocytes. *J Biol Chem*

1984;259:4634-41.

Findlay IA. The Movement of the Mandibular Intra-articular Disc. *Proc R Soc Med* 1965;58;671-5

Fisher E, McLennan SV, Tada H, Heffernan S, Yue DK, Turtle JR. Interaction of Ascorbic Acid and Glucose on the Production of Collagen and Proteoglycan by Fibroblasts. *Diabetes* 1991;40(3):371-6.

Fleischmajer R, Fisher LW, MacDonald ED, Jacobs L Jr. Decorin Interacts with Fibrillar Collagen of Embryonic and Adult Human Skin. *J Struct Biol* 1991;106(1):82-90.

Fransson LÅ. Structure and Function of Cell Associated Proteoglycans. *Trends Biochem Sci* 1987;12:40.

Fransson LÅ, Carlstedt I, Cöster L, Malmström A. *J Biol Chem* 1983;258:14342-45.

Franzen A. Proteoglycans in Skeletal Tissues. A Study of the Molecular Events in Developing Joint Structures. *Doctoral Dissertation.*, Lund University., 1984.

Franzen A, Bjornsson S, Heinegärd D. Cartilage Proteoglycan Aggregate Formation. Role of Link Protein. *Biochem J* 1981;197(3):669-74.

Freshney RI. *Culture of Animal Cells.* 2nd Ed., New York., John Wiley and Sons Inc., 1987.

Fricton JR, Kroening RJ, Hathaway KM. *TMJ and Craniofacial Pain: Diagnosis and Management.* 1st Ed., St. Louis, MO., Ishiyaku Euro-America, Inc., 1988.

Frommer J, Monroe CW. Development and Distribution of Elastic Fibres in the Mandibular Joint of the Mouse, A Comparison of Fetal, Suckling, Juvenile and Adult Stages. *Anat Rec* 1966;156:333-46.

Front P, Aprile F, Mitrovic DR, Swann DA. Age-Related Changes in the Synthesis of Matrix Macromolecules by Bovine Cartilage. *Connect Tiss Res* 1989;19(2-4):121-33.

Fujita S, Hoshino K. Histochemical and Immunohistochemical Studies on the Articular Disk of the Temporomandibular Joint in Rats. *Acta Anat* 1989;134:26-30.

Furstman L. The Early Development of the Human Temporomandibular Joint. *Am J*

Orthod 1963;49(9):672-82.

Furstman L. Normal Age Changes in the Rat Mandibular Joint. *J Dent Res* 1966;45:291.

Gallagher JT, Lyon M, Steward WP. Structure and Function of Heparin Sulfate Proteoglycans. *Biochem J* 1986;236(2):313-25.

Gazit D, Ehrlich J, Kohen Y, Bab I. Effect of Occlusal (Mechanical) Stimulus on Bone Remodelling in Rat Mandibular Condyle. *J Oral Pathol* 1987;16:395.

Ghosh P, Taylor TK. The Knee Joint Meniscus. A Fibrocartilage of Some Distinction. *Clin Orthop* 1987;224:52-63.

Gianelly AA. Orthodontics, Condylar Position, and TMJ Status. *Am J Orthod Dentofac Orthop* 1989;95(6):521-3.

Gianelly AA, Petras JC, Boffa J. Conylar Position and Class II Deep-Bite, No-Overjet Malocclusions. *Am J Orthod Dentofac Orthop* 1989;96(5):428-32.

Gibson GJ, Pearson CH. Sulfated Galactosaminoglycans of Bovine Periodontal Ligament. Evidence for the Presence of Two Major Types of Hybrid But No Chondroitin Sulfate. *Connect Tiss Res* 1982;10:161-171.

Glant TT, Mikecz K, Poole AR. Monoclonal Antibodies to Different Protein-Related Epitopes of Human Articular Cartilage Proteoglycans. *Biochem J* 1986;234(1):31-41.

Glant TT, Mikecz K, Roughley PJ, Buzás E, Poole RA. Age-Related Changes in Protein-Related Epitopes of Human Articular-Cartilage Proteoglycans. *Biochem J* 1986;236(1):71-75.

Glasstone S. Differentiation of the Mouse Embryonic Mandible and Squamo-Mandibular Joint in Organ Culture. *Arch Oral Biol* 1971;16:723.

Glaros AG, Rao SM. Bruxism: A Critical Review. *Psychol Bull* 1977;34(4):767-781.

Glaros AG, Rao SM. Effects of Bruxism: A Review of the Literature. *J Prosth Dent* 1977;38(2):149-57.

Goldberg RL. Enzyme-Linked Immunosorbent Assay for Hyaluronate Using Cartilage Proteoglycan and an Antibody to Keratan Sulfate. *Analyt Biochem* 1988;174:448-58.

Goldman J. Soft Tissue Trauma of the TMJ. In: *Textbook of Temporomandibular Disorders*. Kaplan A, Assael L. (Eds.)., Philadelphia., W.B. Saunders Co., 1991.

Gomori G. Aldehyde-Fuchsin - A New Stain for Elastic Tissue. *American Journal of Clinical Pathology*. 1950;20:665.

Granda JL, Lande MA, Karvonen RL. A Human Cartilage Metalloproteinase with Elastolytic Activity. *Connect Tiss Res* 1990;24(3-4):249-63.

Granström G, Linde A. Glycosaminoglycans of Temporomandibular Articular Discs. *Scand J Dent Res* 1973;81:462-6.

Greaves WS. The Jaw Level System in Ungulates: A New Model. *J Zool* 1978;184:271.

Greene CS. Orthodontics and Temporomandibular Disorders. *Dent Clin North Am* 1988;32(3):529-38.

Greene CS, Laskin DM. Long-Term Status of TMJ Clicking in Patients with Myofacial-Pain and Dysfunction. *J Am Dent Assoc* 1988;117:461-5.

Gressner AM, Schafer S. Comparison of Sulphated Glycosaminoglycan and Hyaluronate Synthesis and Secretion in Cultured Hepatocytes, Fat Storing Cells, and Kupffer Cells. *J Clin Chem Clin Biochem* 1989;27(3):141-9.

Greve H, Blumberg P, Schmidt G, Schlumberger W, Rauterberg J, Kresse H. Influence of Collagen Lattice on the Metabolism of Small Proteoglycan II by Cultured Fibroblasts. *Biochem J* 1990;269(1):149-55.

Griffin CJ, Sharpe CJ. The Structure of the Adult Temporomandibular Meniscus. *Aust Dent J* 1960;5:190-5.

Habuchi H, Kimata K, Suzuki S. Changes in Proteoglycan Composition During Development of Rat Skin. The Occurrence in Fetal Skin of a Chondroitin Sulfate Proteoglycan with a High Turnover Rate. *J Biol Chem* 1986;261(3):1031-40.

Habuchi H, Yamagata T, Iwata H, Sawata S. The Occurance of a Wide Variety of Dermatan Sulfate-Chondroitin Sulfate Copolymers in Fibrous Cartilage. *J Biol Chem* 1973;248(17):6019-28.

Hallen A. Hexosamine and Ester Sulphate Content of the Human Nucleus Pulposis at Different Ages. *Acta Chem Scand* 1958;12:1869.

Hansson T, Öberg T. Arthrosis and Deviations in Form in the Temporomandibular Joint. A Macroscopic Study on Human Autopsy Material. *Acta Odont Scand* 1977;35:167.

Hansson T, Öberg T, Carlsson G, Kopp S. Thickness of Soft Tissue Layers and the Articular Disc of the TMJ. *Acto Odont Scand* 1977;35:77-83.

Hardingham TE. The Role of Link Protein in the Structure of Cartilage Proteoglycan Aggregates. *Biochem J* 1979;177:237-47.

Hardingham TE. Proteoglycans: Their Structure, Interactions and Molecular Organization in Cartilage. *Biochem Soc Trans* 1981;9:489-97.

Hardingham TE, Bayliss M. Proteoglycans of Articular Cartilage: Changes in Aging and in Joint Disease. *Semin Arthritis Rheum* 1990;20(3 Suppl 1):12-33.

Hardingham TE, Beardmore-Gray M, Dunham DG, and Ratcliffe A. Cartilage Proteoglycans. In: *Functions of the Proteoglycans.* Ciba Foundation Symposium., Chichester., Wiley., 1986.

Hardingham TE, Burditt L, Ratcliffe A. Studies on the Synthesis, Secretion, and Assembly of Proteoglycan Aggregates by Chondrocytes. In: *Matrices and Cell Differentiation.* New York., Alan R. Liss Inc., 1984.

Hardingham TE, Muir H. The Specific Interaction of Hyaluronic Acid with Cartilage Proteoglycans. *Biochem Biophys Acta* 1972a;279:401-5.

Hardingham TE, Muir H. Binding of Oligosaccharides of Hyaluronic Acid to Proteoglycans. *Biochem J* 1973;135:905-8.

Harpman JA, Woollard HH. The Tendon of the Lateral Pterygoid Muscle. *J Anat* 1938;73:112-5.

Harris E. Biology of the Joint. In: *Textbook of Rheumatology.* Kelly WH, Harris ED, Ruddy S, Sledge CB. (Eds.)., Philadelphia., W.B. Saunders Co., 1985.

Hascall VC. Introduction. In: *Function of the Proteoglycans.* Ciba Foundation Symposium. 1986;124:1-8.

Hascall VC, Hascall GK. Proteoglycans. In: *Cell Biology of the Extracellular Matrix.* Hay ED. (Ed.)., New York., Plenum., 1981.

Hascall VC, Heinegärd DK. Aggregate of Cartilage Proteoglycans. II. Oligosaccharide Competitors of the Proteoglycan-Hyaluronic Acid Interactions. *J Biol Chem* 1974;249:4242-9.

Hascall VC, Riolo RL. Characteristics of the Protein-Keratan Sulphate Core of the Keratan Sulfate Prepared from the Bovine Nasal Cartilage. *J Biol Chem* 1972;247(14):4529-8.

Hascall VC, Sajdera SW. Physical Properties and Polydispersity of Proteoglycan from Bovine Nasal Cartilage. *J Biol Chem* 1970;245:4920-30.

Hata R, Nagai Y. A Rapid and Micro Method for Separation of Acidic Glycosaminoglycans by Two-Dimensional Electrophoresis. *Analyt Biochem Biophys* 1972;41:67-9.

Hatcher DC, Faulkner MG, Hay A. Development of Mechanical and Mathematical Models to Study Temporomandibular Joint Loading. *J Prost Dent* 1986;55(3):377-84.

Hay ED. *Cell Biology of the Extracellular Matrix.* Hay ED. (Ed.)., New York., Plenum., 1981.

Heffez L, Jordan S. A Classification of Temporomandibular Disk Morphology. *Oral Surg Oral Med Oral Pathol* 1989;67(1):11-9.

Heinegärd D, Axelsson I. The Distribution of Keratan Sulphate in Cartilage Proteoglycans. *J Biol Chem* 1977;252:1971-9.

Heinegärd D, Bjorne-Persson A, Coster L, Franzen A, Gardell S. The Core Proteins of Large and Small Interstitial Proteoglycans from Various Connective Tissues from Distinct Subgroups. *Biochem J* 1985;230(1):181-94.

Heinegärd D, Frazen A, Hedbom E, Sommarin Y. Common Structures of the Core Proteins of Interstitial Proteoglycans. In: *Functions of the Proteoglycans.* Ciba Foundation Symposium., Chichester., Wiley., 1986;124:69.

Heinegärd DK, Hascall VC. Aggregation of Cartilage Proteoglycans. III. Characteristics of the Proteins Isolated from Trypsin Digest of Aggregates. *J Biol Chem* 1974;249:4250-6.

Heinegärd DK, Hascall VC. The Effect of Dansylation and Acetylation on the Interaction Between Hyaluronic Acid Binding Region of Cartilage Proteoglycans. *J Biol Chem* 1979;254:921-6.

Heinegärd D, Oldberg A. Structure and Biology of Cartilage and Bone Matrix Noncollagenous Macromolecules. *FASEB J* 1989;3:2042-51.

Heinegärd D, Paulsson M. Structure and Metabolism of Proteoglycans. In: *Extracellular Matrix Biochemistry*. Piez KA, Reddi AH (Eds.)., Amsterdam., Elsevier Science Publishing Co. Inc., 1984.

Hellsing RJ, Holmlund A. Development of Anterior Disc Displacement in the Temporomandibular Joint: An Autopsy Study. *J Prosthet Dent* 1985;53:397.

Herbosa EG, Rotskoff KS, Ramos BF, Ambrookian HS. Condylar Position in Superior Maxillary Repositioning and its Effect on the Temporomandibular Joint. *J Oral Maxillofac Surg* 1990;49(7):690-6.

Hinton RJ. Form and Function of the Temporomandibular Joint. In: *Craniofacial Biology*., Craniofacial Growth Series., Monograoh #10., Carlson DS. (Ed.)., Ann Arbor., University of Michigan., Center for Human Growth and Development., 1981;37-60.

Hirschmann PN, Shuttleworth CA. The Collagen Composition of the Mandibular Joint of the Foetal Calf. *Archs Oral Biol* 1976;21:771-3.

Hjortsjo CH. The Significance of the Articular Disc and the Accentuated Grinding Joint. *Odontologisk Revy* 1953;4:203-9.

Hoffman P, Linker A, Meyer K. Uronic Acid of Chondroitin Sulfate B. *Science* 1956;124:1252.

Honda T, Katagari K, Kuroda A, Matsunaga E, Shinkai H. Age-Related Changes of the Dermatan Sulfate Containing Small Proteoglycans in Bovine Tendon. *Coll Relat Res* 1987;7(3):171-84.

Höök M, Kjellen L, Johansson S, Robinsson J. Cell Surface Glycosaminoglycans. *Annu Rev Biochem* 1984;53:847-69.

Höök M, Lindahl U, Bäckström G, Malmström A, Fransson LÅ. *J Biol Chem* 1974;249:3908-15.

Horowitz MI, Pigman W. The Glycoconjugates, Volume II: Mammalian Glycoproteins, Glycolipids, and Proteoglycans. Horowitz MI, Pigman W. (Eds.)., New York., Academic Press., 1978.

Howard J. Reductive Mobilization of the Acute Closed Lock in the Temporomandibular Joint. In: Diagnosis of Internal Derangements of the Temporomandibular Joint. Vol. 1., Moffet BC. (Ed.)., Seattle., University of Washington., 1984.

Huber P. Junk Science in the Court Room. *Forbes* 1991;July 8:68-72.

Huey G, Stair S, Stern R. Hyaluronic Acid Determinations: Optimizing Assay Parameters. Matrix 1990;10:67-74.

Huey G, Moiin A, Stern R. Levels of [^{3}H]Glucosamine Incorporation into Hyaluronic Acid by Fibroblasts is Modulated by Culture Conditions. Matrix 1990;10:75-83.

Hukins DWL. *Connective Tissue Matrix.* London., MacMillan., 1984;1-160.

Hunziker EB, Schenk RK. Structural Organization of Proteoglycans in Cartilage. In: *Biology of Proteoglycans.* Wight TN, Mechan RP. (Eds.)., London., Academic Press., 1987.

Hylander WL. Mandibular Function and TMJ Loading. *In* Carlson DS, McNamara JA, Ribbens KA (Eds.). *Developmental Aspects of Temporomandibular Joint Disorders.* Monograph #16. Craniofacial Growth Series. Center for Human Growth and Development, University of Michigan, Ann Arbor, MI, 1985b.

Hylander WL. Functional Anatomy. *In* Sarnat BG, Laskin DM (Eds.). *The Temporomandibular Joint: A Biological Basis for Clinical Practice.* 4th Ed, Philadelphia, W.B. Saunders Company, 1992.

Ito T, Gibbs CH, Marguelles-Bonnet R, Lupkiewicz SM, Young HM, Lundeen HC, Mahan PE. Loading on the Temporomandibular Joints with Five Occlusal Conditions. *J Prosth Dent* 1986;56(4):478-84.

Inerot S, Heinegärd D, Audell L, Olsson SE. Articular-Cartilage Proteoglycans in Aging and Osteoarthritis. *Biochem J* 1978;169:143.

Ingman AM, Ghosh P, Taylor TKF. Variation of Collagenous and Non-collagenous Proteins of Human Knee Menisci with Age and Degeneration. *Gerontologia* 1974;20:212-23.

Ingervall B, Carlsson GE, Thilander B. Postnatal Development of the Human Temporomandibular Joint: II. A Microradiographic Study. *Acta Odont Scand* 1976;34:133-9.

Jackson RL, Busch SJ, Cardin AD. Glycosaminoglycans: Molecular Properties, Protein Interactions, and Role in Physiological Processes. *Physiological Reviews* 1991;71(2):481-539.

Jaffe EA. Endothelial Cells and the Biology of Factor VIII. *New England Journal of Medicine* 1977;296:377.

Jagger RG. The Surface Structure of the Temporomandibular Joint Disk: A Scanning Electron Microscope Study. *J Oral Rehab* 1980;7:225.

Jee W. The Skeletal Tissues. In: *Histology, Cell and Tissue Biology.* Weiss L (ed.)., New York., Elsevier Biomedical Publishers., 1988.

Jennings J, Buchanan F, Freeman D, Garland JT. *J Clin Endocrinol Metab* 1980;51:1166-70.

Kahari VM, Larjava H, Uitto J. Differential Regulation of Extracellular Matrix Proteoglycan (PG) Gene Expression. *J Biol Chem* 1991;266(16):10608-15.

Kahn A, Pottenger LA, Phillips FM, Viola RW. Evidence of Proteoglycan/ Proteoglycan Interactions within Aggregates. *J Orthop Res* 1991;9(6):777-86.

Kantomaa T. The Effect of Increased Displacement of the Glenoid Fossa on Mandibular Growth: A Methodological Study in the Rabbit. *Eur J Ortho* 1984a;6:15.

Kantomaa T. The Effect of Increased Upward Displacement of the Glenoid Fossa on Mandibular Growth. *Eur J Ortho* 1984b;6:183.

Kantomaa T. Effect of Functional Change on Cell Differentiation in the Condylar Cartilage. *J Anat* 1987;152:133-143.

Kantomaa T, Hall BK. Mechanism of Adaptation in the Mandibular Condyle of the Mouse. An Organ Culture Study. *Acta Anat* 1988;132:114.

Kao J, Huey G, Kao R, Stern R. Ascorbic Acid Stimulates Production of Glycosaminoglycans in Cultured Fibroblasts. *Exp Mol Pathol* 1990;53(1):1-10.

Kaplan AS, Assael LA. *Temporomandibular Disorders: Diagnosis and Treatment.* Philadelphia., W.B. Saunders Company., 1991.

Kato Y, Gospodarowicz D. Sulfated Proteoglycan Synthesis by Confluent Cultures of Rabbit Costal Chondrocytes Grown in the Presence of Fibroblast Growth Factor. *J Cell*

Biol 1985a;100(2):477-85.

Kato Y, Gospodarowicz D. Effect of Exogenous Extracellular Matrices on Proteoglycan Synthesis by Cultured Rabbit Costal Chondrocytes. *J Cell Biol* 1985b;100(2):486-95.

Kato Y, Gospodarowicz D. Stimulation by Glucocorticoid of the Synthesis of Cartilage Matrix Proteoglycans Produced by Rabbit Costal Chondrocytes *in vitro*. *J Biol Chem* 1985c;260:2364-73.

Katz M, Kvinnsland S. Matrix Formation of the Mandibular Condyle of the Rat. [^{35}S]-sulphate Incorporation Studies. *Acta Odont Scand* 1979;37:137.

Katzberg RW. Temporomandibular Joint Imaging. *Radiology*. 1989;170:297.

Keiser HD. A Solid-Phase Immunoassay for the Binding of Cartilage Proteoglycan to Hyaluronic Acid. *Analyt Biochem* 1987;160:462-67.

Keiser HD, Diamond BA. Monoclonal Antibodies Reactive with Keratan Sulphate-bearing Tryptic Fragments of Bovine Nasal Cartilage Proteoglycan. *Connect Tiss Res* 1987;16(2):131-41.

Keiser HD, Schulman HJ, Sandson JI. Immunochemistry of Cartilage Proteoglycans: Immunodiffusion and Gel-Electrophoretic Studies. *Biochem J* 1972;126:163-9.

Keith DA. Elastin in the Bovine Mandibular Joint. *Arch Oral Biol* 1979;24:211-5.

Keith DA. Development of the Human Temporomandibular Joint. *Br J Oral Surg* 1982;20:217-24.

Kempson GE, Muir H, Swanson SAV, Freeman MAR. Correlations Between Stiffness and the Chemical Constituents of Cartilage on the Human Femoral Head. *Biochim Biophys Acta* 1970;(215):70-77.

Kimura JH, Caputo CB, Hascall VC. The Effect of Cycloheximide on Synthesis of Proteoglycans by Cultured Chondrocytes from Swarm Rat Chondrosarcoma. *J Biol Chem* 1981a;256:4368-76.

Kimura JH, Hardingham TE, Hascall VC. Assembly of Newly Synthesized Proteoglycan and Link Protein into Aggregates in Cultures of Chondrocytes from the Swarm Rat Chondrosarcoma. *J Biol Chem* 1980;255:7134-43.

Kimura JH, Hardingham TE, Hascall VC, Solursh M. Biosynthesis of Proteoglycans and their Assembly into Aggregates in Cultures of Chondrocytes from the Swarm Rat Chondrosarcoma. *J Biol Chem* 1979;254:2600-9.

Kimura JH, Lohmander LS, Hascall VC. Studies on the Biosynthesis of Cartilage Proteoglycan in a Model System of Cultured Chondrocytes from the Swarm Rat Chondrosarcoma. *J Cell Biol* 1984;26:261-78.

Kimura JH, Thonar EJMA, Hascall VC, Reiner A, Poole AR. Identification of Core Protein, an Intermediate in Proteoglycan Biosynthesis in Cultured Chondrocytes from the Swarm Rat Chondrosarcoma. *J Biol Chem* 1981b;256:7890-97.

Kittlick PD. Glycosaminoglycans. Recent Biochemical Results in the Fields of Growth and Inflammation. *Exp Path Suppl* 1985;10:1-174.

Kjellan L, Lindahl U. Proteoglycans: Structures and Interactions. *Annu Rev Biochem* 1991;60:443-75.

Klein-Nulend J, Veldhuijzen JP, van de Stadt RJ, van Kampen GP, Kuijer R, Burger EH. Influence of Intermittent Compressive Force on Proteoglycan Content in Calcifying Growth Plate Cartilage *in Vitro*. *J Biol Chem* 1987;262(32):15490-5.

Knudson W, Toole BP. Membrane Association of the Hyaluronate Stimulatory Factor for LX-1 Human Lung Carcinoma Cells. *J Cell Biochem* 1988;38:165-77.

Kongtawelert P, Ghosh P. An Enzyme-Linked Immunosorbent-Inhibition Assay for Quantification of Hyaluronan (Hyaluronic Acid) in Biological Fluids. *Analyt Biochem* 1989;178:367-72.

Kosiagin DV. The Ratio of Protein and Carbohydrate Components of Proteoglycans from Normal and Degenerative Articular Cartilage. *Ukr Biokhim Zh* 1986;58(3):65-8.

Koski K, Ronning O. Growth Potential of Suncutaneously Transplanted Cranial Base Synchondroses of the Rat. *Acta Odont Scand* 1969;27:343.

Koski K, Ronning O. Condyle Neck Periostomy and the Mitotic Activity in the Condylar Tissue in the Young Rats. *Swed Dent J* (Suppl.), 1982;15:109.

Kopp S. Topographical Distribution of Sulphated Glycosaminoglycans in Human Temporomandibular Joint Disks: A Histochemical Study of an Autopsy Material. *J Oral Path* 1976;5:265-76.

Kosakai M, Yosizawa Z. A Partial Modification of the Carbazole Method of Bitter and Muir for Quantitation of Hexuronic Acids. *Analyt Biochem* 1979;93:295-8.

Kresse H, Glossl J, Hoppe W, Rauch U, Quentin E. Biosynthesis and Processing of Proteodermatan Sulfate. In: *Functions of the Proteoglycans.* Ciba Foundation Symposium., Chichester., Wiley., 1986.

Krogh-Poulsen W, Molhave A. Om Discus Articulationis Tempormandibularis. *Tandlaegebladet* 1957;61;265-71

Krusius T, Ruohslahti E. Primary Structure of Extracellular Matrix Proteoglycan Core Deduced From Cloned cDNA. *Proc Natl Acad Sci* USA 1986;83(20):7683-7.

Kurita K, Westesson PL, Sternby NH, Eriksson L, Carlsson LE, Lundh H, Toremalm NG. Histologic Features of the Temporomandibular Joint Disk and Posterior Disk Attachment: Comparison of Symptom-free Persons with Normally Positioned Disks and Patients with Internal Derangement. *Oral Surg Oral Med Oral Path* 1989;67(6):635-43.

Last KS, Embery G. Hyaluronic Acid and Hyaluronidase Activity in Gingival Exudate From Sites of Acute Ulcerative Gingivitis in Man. *Archs Oral Biol* 1987;32(11):811-15.

Laurent TC, Fraser JRE. The Properties and Turnover of Hyaluronan. In: *Functions of the Proteoglycans, CIBA Foundation Symposium.* Evered D, Whelan J. (Eds.)., New York., Wiley., 1986.

Laurent TC, Wasteson Å, Öbrink B. Macromolecular Properties of Glycosaminoglycans (Mucopolysaccharides) and Proteoglycans. In: *Aging of Connective and Skeletal Tissue.* Stockholm., Nordiska Bokhandelns Förlag., 1969;65-80.

Laurent UGB, Tengblad A. Determination of Hyaluronate in Biological Samples by a Specific Radioassay Technique. *Analyt Biochem* 1980;109:386-94.

Lekkas C, Honee GLJM, Van Den Hööf A. Effects of Experimental Defects of the Articular Disc of the Temporomandibular Joint in Rats. *J Oral Rehab* 1988;15:141.

Lemperg R, Larsson EE, Hjertquist SO. The Glycosaminoglycans of the Bovine Articular Cartilage. I. Concentration and Distribution in Different Layers in Relation to Age. *Calcif Tissue Res* 1974;15(3):237-51.

Lev R, Spicer SS. Specific Staining of Sulphate Groups with Alcian Blue at Low pH. *J Histochem Cytochem* 1964;12:309.

Levy BM. Growth of the Mandibular Joint in Normal Mice. *JADA* 1949;36:177.

Lewandowska K, Choi HU, Rosenberg LC, Sasse Jm Neame PJ. Extracellular Matrix Adhesion Proteoglycan-Associated Protein (22K) from Bovine Fetal Skin. *J Cell Sci* 1991;99(Pt.3)657-68.

Ling GN. The Association-Induction Hypothesis I. Association of Ions and Water with Macromolecules. In: *In Search of the Physical Basis of Life*. New York., Plenum, 1984

Lippiello L, Yamamoto K, Robinson D, Mankin H. Involvement of Prostaglandins from Rheumatoid Synovium in Inhibition of Articular Cartilage Metabolism. *Arthritis Rheum* 1978;21:909-17.

Lipshitz H. Fluid Exudation and Load-Deformation Properties of Articular Cartilage During Compression. In: *Water in Polymers*. Rowland SP (Ed.). Washington DC,. American Chemical Society, 1980.

Luder HU, Schroeder HE. Light and Electron Microscopic Morphology of the Temporomandibular Joint in Growing and Mature Crab-Eating Monkeys (Macaca Fascicularis): The Condylar Articular Layer. *Anat Embryol Berl* 1990;181(5):499-511.

Lundh H, Westesson PL, Kopp S. A Three-Year Follow-Up of Patients with Reciprocal Temporomandibular Joint Clicking. *Oral Surg Oral Med Oral Pathol.* 1987;63(5):530-3.

MacConail MA. The Movements of Bones and Joints. The Synovial Fluid and its Assistants. *J Bone Joint Surg* 1950;32B:244-52.

Mahan PE. Seminar on TMJ Disorders and Facial Pain. *Northeast Dental Seminars.* Boston., Dec. 11-12., 1985.

Mahdy AE. Intra-articular Tissue in the Temporomandibular Joint. *J Prosthet Dent* 1971;26(4);396-405.

Malemud CJ, Papay RS. Rabbit Chonrocytes Maintained in Serum-Free Medium. I. Synthesis and Secretion of Hydrodynamically-Small Proteoglycans. *Exp Cell Res* 1986;167(2):440-52.

Malemud CJ, Sokoloff L. The Effect of Prostaglandins on Cultured Lapine Articular Chondrocytes. *Prostaglandins* 1977;13:845-60.

Mallinger R, Stockinger L. Histochemistry of the Extracellular Matrix of Aging

Hyaline Cartilage. *Folia Histochem Cytobiol* 1987;25(2):129-32.

Mankin H, Radin E. Structure and Function of Joints. In: *Arthritis and Allied Conditions.*, 10th Ed., McCarty D (Ed.)., Philadelphia., Lea and Febiger., 1985.

Maroudas A. Transport Through Articular Cartilage and Some Physiological Implications. In: *Normal and Osteoarthritic Articular Cartilage.* Ali SY, Elves MW, Leaback DM.(Eds.)., London., Institute of Orthopedics., 1974.

Maroudas A. Physicochemical Properties of Articular Cartilage. In: *Adult Articular Cartilage.* Freeman MAR. (Ed.)., England., Pitman., 1979;215-90.

Maroudas A. Physical Chemistry of Articular Cartilage and the Intervertebral Disc. In: *The Joints and Synovial Fluid.* Sokoloff. (Ed.)., New York., Academic Press., 1980;95-137.

Maroudas A. Proteoglycan Osmotic Pressure and Collagen Tension in Normal, Osteoarthritic Human Cartilage. *Osteoarthrit Symp* 1981;36-8.

Maroudas A, Evans H. Sulphate Diffusion and Incorporation into Human Articular Cartilage. *Biochem Biophys Acta* 1974;338:265.

Maroudas A, Evans H, Almeida L. Cartilage of the Hip Joint. Topographical Variations of Glycosaminoglyan Content in Normal and Fibrillated Tissue. *Ann Rheum Dis* 1973;32(1):1-9.

Maroudas A, Muir H, Wingham J. The Correlation of the Fixed Negative Charge with Glycosaminoglycan Content of Human Articular Cartilage. *Biochem Biophys Acta* 1969;177(3):492-500.

Maroudas A, Ziv I, Weisman N, Venn M. Studies of Hydration and Swelling Pressure in Normal and Osteoarthritic Cartilage. *Biorheology* 1985;22:159.

Masson P. Some Histologic Methods. Trichrome Stainings and their Preliminary Technique. *Bulletin of the International Association of Medicine.* 1929;12:75.

Maukin HJ, Radin E. Structure and Function of Joints. In: *Arthritis and Allied Conditions.* 10th ed., McCarty D, Ed., Philadelphia., Lea and Febiger., 1985.

Maukin HJ, Radin E. Structure and Function of Joints. In: *Arthritis and Allied Conditions.* 11th ed., McCarty D, Ed., Philadelphia., Lea and Febiger., 1989.

Markowitz HA, Gerry RG. Temporomandibular Joint Disease. *Oral Surg* 1949;2;1309-37

McDevitt CA. Biochemistry of Articular Cartilage. Nature of Proteoglycan and Collagen of Articular Cartilage and their Role in Aging and Osteoarthrosis. *Ann Rheum Dis* 1973;32:364-78.

McDevitt CA, Billingham M, Muir H. *In vivo* Metabolism of Proteoglycans in Experimental Osteoarthritic and Normal and Canine Articular Cartilage and Intervertebral Disc. *Semin Arthritis Rheum* (Supplement) 1981;11:17-8.

McDevitt CA, Webber RJ. The Ultrastructure and Biochemistry of Meniscal Cartilage. *Clin Orthop* 1990;252:8-18.

McDonald F. The Condylar Disk as a Controlling Factor in the Form of the Condylar Head. *J Craniomandib Disord Facial Oral Pain* 1989;3(2):83-86.

McNamara JA. The Independent Functions of the Two Heads of the Lateral Pterygoid Muscle. *Am J Anat* 1973;138:197.

McNamara JA, Carlsson DS. Quantitative Analysis of Temporomandibular Joint Adaptations to Protrusive Function. *Am J Orthod* 1979;76:593.

McNamara JA, Connelly TG, McBride MC. Histological Studies of Temporomandibular Joint Adaptations. In: *Determinants of Mandibular Form and Growth.* McNamara JA (Ed.)., Monograph #4, Craniofacial Growth Series., Center for Human Growth and Development., The University of Michigan., Ann Arbor., Michigan., 1975.

McNamara JA, McBride MC. A Histologic Study of the Post-Natal Development of the Temporomandibular Articulation in *Macaca mulatta. Anat Rec* 1974;178:408.

Mechanic G. Collagen Biochemistry. In: *Skeletal Research: An Experimental Approach.* Simmons DJ, Kunin AS. (Eds.)., New York., Academic Press., 1979:227-41.

Melching LI, Roughley PJ. The Synthesis of Dermatan Sulphate Proteoglycans by Fetal and Adult Human Articular Cartilage. *Biochem J* 1989;261(2):501-8.

Meyer K, Davidson E, Linker A, Hoffman P. *Biochim Biophys Acta* 1956;(21):506.

Miles AEW, Dawson JA. Elastic Fibres in the Articular Fibrous Tissue of Some Joints. *Arch Oral Biol* 1962;7:249-52.

Mills ER, Daniel JC, Scapino R. Histologic Features and *In Vitro* Proteoglycan Synthesis in the Rabbit Craniomandibular Joint Disk. *Arch Oral Biol* 1988;33:195-202.

Mitrovic D, Lippiello L, Gruson F, Aprile F, Mankin H. Effects of Various Prostanoids on the *In Vitro* Metabolism of Bovine Articular Chondrocytes. *Prostaglandins* 1981;22:499-511.

Moffett BC. The Prenatal Development of the Human Temporomandibular Joint. *Contrib to Embryol* 1957;36:19-28.

Moffett BC. The Morphogenesis of the Temporomandibular Joint. *Am J Orthod* 1966;52(6):401-15.

Moffett BC. Definitions of Temporomandibular Joint Derangements. *In: Diagnosis of Internal Derangements of the Temporomandibular Joint*. Moffett BC (Ed). Vol 1. Seattle. University of Washington. 1984a.

Moffett BC. Histological Aspects of Temporomandibular Joint Derangements. *In: Diagnosis of Internal Derangements of the Temporomandibular Joint*. Moffett BC (Ed). Vol 1. Seattle. University of Washington. 1984b.

Mohl N. The Temporomandibular Joint. In: *A Textbook of Occlusion.* Mohl N *et al.* (Eds.)., Lombard IL., Quintessence Publishing Co., 1988.

Moore KL. *The Developing Human* 4th Ed. Philadelphia., W. B. Saunders Co., 1989.

Motohashi N, Mori I. Quantitation of Hyaluronic Acid and Chondroitin Sulphates in Rabbit Synovial Fluid by High-Performance Liquid Chromatography of Oligosaccharides Enzymatically Derived Thereof. *Chem Pharm Bull* 1990;38(3):769-73.

Morimoto K, Hashimoto N, Suetsugu T. Prenatal Developmental Process of Human Temporomandibular Joint. *J Prosthet Dent* 1987;57(6):723-30.

Moses JJ, Topper DC. A Functional Approach to the Treatment of Temporomandibular Joint Internal Derangement. *J Craniomandib Disord Facial Oral Pain* 1991;5(1):19-27.

Muir H. Proteoglycans: State of the Art. *Osteo Symposium.* 1981, pp7-10.

Murray PDF, Drachman DB. The Role of Movement in the Development of Joints and Related Structures: the Head and Neck in the Chick Embryo. *J Embyrol Exp Morphol* 1969;22(3):349-68.

Myers ER, Armstrong CG, Mow VC. Swelling Pressure and Collagen Tension. In: *Connective Tissue Matrix.* Hukins DWL. (Ed.)., London., McMillan., 1984;161-86.

Myers ER, Mow VC. Biomechanics of Cartilage and its Response to Biomechanical Stimuli. In: *Cartilage, Vol 1: Structure, Function, and Biochemistry.* Hall BK (Ed.)., New York., Academic Press., 1983.

Myers ER, Zhu W, Mow VC. Viscoelastic Properties of Articular Cartilage and Meniscus. In: *Collagen, Volume II, Biochemistry and Biomechanics.* Nimni ME.,Florida., CRC Press Inc., 1988:267-88.

Nachman R, Levine R, Jaffe E. Synthesis of Factor VIII Antigen by Cultured Guinea Pig Megakaryocytes. *Clinical Research* 1977;25:479.

Nadji M, Gonzales MS, Castro A. Factor VIII Related Antigen: An Endothelial Cell Marker. *Laboratory Investigation* 1980;42:139.

Nagy NB, Daniel JC. Distribution of Elastic Fibres in the Developing Rabbit Craniomandibular Joint. *Arch Oral Biol* 1991;36(1):15-23.

Nakanishi T, Iwai-Liao Y. Comparative Histological Contributions to the Development of the Ear-Ossicular Joints and the Temporomandibular Joint in the Mouse. *Okajimas Folia Anat Jpn* 1990;67(5):381-91.

Nakano T, Scott PG. A Quantitative Chemical Study of Glycosaminoglycans in the Articular Disc of the Bovine Temporomandibular Joint. *Arch Oral Biol* 1989;34(9):749-57.

Nakano T, Scott PG. Proteoglycans of the Articular Disc of the Bovine Temporomandibular Joint. I. High Molecular Weight Chondroitin Sulfate Proteoglycan. *Matrix* 1989;9(4):277-83.

Neame PJ, Choi HU, Rosenberg LC. The Primary Structure of the Core Protein of the Small, Leucine-rich Proteoglycan (PG I) from Bovine Articular Cartilage. *J Biolog Chem* 1989;264(15):8653-61.

Nickel JC, McLachlan KR, Smith DM. Eminence Development of the Postnatal Human Temporomandibular Joint. *J Dent Res* 1988a;67:896-902.

Nickel JC, McLachlan KR, Smith DM. A Theoretical Model of Loading and Eminence Development of the Postnatal Human Temporomandibular Joint. *J Dent Res* 1988b;67(6):903-10.

Nishiyama H. Biochemical and Immunological Study of Lumbar Disc. *Nippon Seikeigeka Gakkai Zasshi* 1985;59(12):1119-31.

Norkin C, Levangie P. *Joint Structure and Function.* Philadelphia., F.A. Davis., 1983.

Öberg T, Carlsson GE, Fajers CM. The Temporomandibular Joint. A Morphological Study on Human Autopsy Material. *Acta Odont Scand* 1971;29:349.

Öberg T, Carlsson GE, Fajers CM, Bergman F. Aging of the Temporomandibular Disc With Special Reference to the Occurence of Cartilaginous Cells. *Odontol Foren Tidskr* 1966;74;122-9.

Okeson JP. *Management of Temporomandibular Disorders and Occlusion.* 1st Ed., St. Louis., Mosby., 1985.

Okeson JP. *Management of Temporomandibular Disorders and Occlusion.* 2nd Ed., St. Louis., Mosby., 1989.

Ogus HD, Toller PA. *Common Disorders of the Temporomandibular Joint.* 2nd ed., Bristol., John Wright and Sons Ltd., 1986.

Orban BJ. *Oral Histology and Embryology.* 4th ed., St. Louis., C.V. Mosby, 1957.

Orban BJ. *Oral Histology and Embryology.* 11th ed., St. Louis., C.V. Mosby, 1991.

O'Ryan F, Epker BN. Temporomandibular Joint Function and Morphology: Observations on the Spectra of Normalcy. *Oral Surg* 1984;58(3):272-9.

Osborn JW. The Temporomandibular Ligament and the Articular Eminence as Constraints During Jaw Opening. *J Oral Rehab* 1989;16:323-33.

Osborn JW. The Disc of the Human Temporomandibular Joint: Design, Function and Failure. *J Oral Rehab* 1985;12:279-93.

Otsu K, Inoue H, Tsuzuki Y, Yonekura Y, Nakanishi Y, Suzuki S. *Biochem J* 1985;37-48.

Palacios E, Valvassori GE, Shannon M, Reed CF. *Magnetic Resonance of the Temporomandibular Joint.* New York., Thieme Medical Publishers Inc., 1990.

Paroli E, Antonilli L, Biffoni M. A Pharmacological Approach to Glycosaminoglycans. *Drugs Exp Clin Res* 1991;17(1):9-19.

Pasternack SG, Veis A, Breen M. Solvent Dependant Changes in Proteoglycan Subunit Conformation in Aqeous Guanidine Hydrochloride Solutions. *J Biol Chem* 1974;249:2206-11.

Pearce RH, Thompson JP, Bebault GM, Flak B. Magnetic Resonance Imaging Reflects the Chemical Changes of Aging Degeneration in the Human Intervertebral Disk. *J Rheumatol Suppl* 1991;27:42-3.

Pearson CH, Gibson GJ. Proteoglycans of Bovine Periodontal Ligament and Skin. Occurence of Different Hybrid-Sulphated Glycosaminoglycans in Distinct Proteoglycans. *Biochem J* 1982;210:27-37.

Pearson CH, Pringle GA. Chemical and Immunochemical Characteristics of Proteoglycans in Bovine Gingiva and Dental Pulp. *Archs Oral Biol* 1986;31(8):541-8.

Perry H, Xu Y, Forbes D. The Embryology of the Temporomandibular Joint. *Cranio* 1985;3(2):125-32.

Petrovic A. Experimental and Cybernetic Approaches to the Mechanism of Action of Functional Appliances. In: *Clinical Alteration of the Growing Face.* McNamara JA (Ed.)., Monograph #14.,Craniofacial Growth Series., Center for Human Growth and Development., University of Michigan., Ann Arbor, Michigan., 1985:213-68.

Petrovic A, Stutzman J, Oudet C. Control Processes in the Postnatal Growth of the Mandibular Condylar Cartilage. In: *Determinants of Mandibular Form and Growth.* McNamara JA. (Ed.)., Monograph #4., Craniofacial Growth Series., Center for Human Growth and Development., University of Michigan., Ann Arbor, Michigan., 1975, 101-53.

Pharmacia Fine Chemicals. Ion Exchange Chromatography: Principles and Methods. (Product Information Catalogue)., 1980.

Pieraggi MT, Bouissou H, Angelier C, Uhart D, Magnol JP, Kokolo J. The Fibroblast. *Ann Pathol* 1985;5(2):65-76.

Pollack B. Cases of Note: Michigan Jury Awards $850,000 in Ortho Case: A Tempest in a Teapot. *Am J Orthod Dentofac Orthop* 1988;94:358-9.

Poole AR. Proteoglycans in Health and Disease: Structures and Functions. *Biochem J* 1986;236:1-14

Poole AR, Webber C, Pidoux I, Choi H, Rosenberg LC. Localization of a Dermatan

Sulphate Proteoglycan (DS-PGII) in Cartilage and the Presence of an Immunologically Related Species in Other Tissues. *J Histochem Cytochem* 1986;34(5):619-25.

Pringle GA, Dodd CM. Immunoelectron Microscopic Localization of the Core Protein of Decorin Near the D and E Bands of Tendon Collagen Fibrils by Use of Monoclonal Antibodies. *J Histochem Cytochem* 1990;38(10):1405-11.

Pringle GA, Dodd CM, Osborn JW, Pearson CH, Mosmann TR. Production and Characterization of Monoclonal Antibodies to Bovine Skin Proteodermatan Sulphate. *Collagen Rel Res* 1985;5:23-39.

Prins APA, Lipman JM, McDevitt CA, Sokoloff L. *Arthritis Rheum* 1982;25:1228-38.

Pullinger AC, Seligman DA. TMJ Osteoarthrosis: A Differentiation of Diagnosis and Subgroups by Symptom History and Demographics. *J Craniomand Disord Facial Oral Pain.* 1987;1(4):251-6.

Purvis JA, Embery G, Oliver WM. *Arch Oral Biol* 1984;29:513-9.

Quinn JH. Pathogenesis of Temporomandibular Joint Chondromalacia and Arthralgia. *Oral Maxillofac Surg Clin North Am* 1989;1:47-57.

Ranly DM. *A Synopsis of Craniofacial Growth.* 2nd Ed., Connecticut., Appleton and Lange., 1988.

Rasmussen OC. Description of Population and Progress of Symptoms in a Longitudinal Study of Temporomandibular Arthropathy. *Scand J Dent Res.* 1981;89:196-203.

Ratcliffe A, Doherty M, Maini RN, Hardingham TE. Increased Concentrations of Proteoglycan Components in the Synovial Joints of Patients with Acute but not Chronic Joint Disease. *Ann Rheum Dis* 1988;47(10):826-32.

Ratcliffe A, Fryer PR, Hardingham TE. The Distribution of Aggregating Proteoglycans in Articular Cartilage: Comparison of Quantitative Immonoelectron Microscopy with Radioimmunoassay and Biochemical Analysis. *J Histochem Cytochem* 1984;32:193-201.

Ratcliffe A, Fryer PR, Hardingham TE. Proteoglycan Biosynthesis in Chondrocytes: Protein A-Gold Localization of Proteoglycan Protein Core and Chondroitin Sulfate within Golgi Subcompartments. *J Cell Biol* 1985;101:2355-65.

Ratcliffe A, Tyler JA, Hardingham TE. Articular Cartilage Cultured with Interleukin. I. Increased Release of Link Protein, Hyaluronic Acid Binding Region and Other Proteoglycan Fragments. *Biochem J* 1986;238(2):571-80.

Rayne J. Functional Anatomy of the Temporomandibular Joint. *Br J Oral Max Surg* 1987;25:92-99.

Rees LA. The Structure and Function of the Mandibular Joint. *Br Dent J* 1954;Mar 16;125-33.

Rennard SI, Berg R, Martin GR, Foidart JM, Gehron Robey P. Enzyne-Linked Immunosorbent Assay (ELISA) for connective tissue components. *Anal Biochem* 1980:104:205-14.

Reihanian H, Jamieson AM, Tang LH, Rosenberg L. Hydrodynamic Properties of Proteoglycan Subunit from Bovine Nasal Cartilage. Self-Association Behavior and Interaction with Hyaluronate Studied by Laser Light Scattering. *Biopolymers* 1979;18:1727-47.

Ricketts RM. Occlusion - The Medium of Dentistry. *J Prosth Dent* 1969;21(1):39-60.

Robert L. Le Vieillissement du Tissu Conjonctif. Aspects Moleculaires et Cellulaires. *Phlebologie* 1986;39(4):783-90.

Robinson DG, Ehlers U, Herkin R, Herrmann B, Mayer F, Schurmann FW. *Methods of Preparation for Electron Microscopy: An Introduction for the Biomedical Sciences.* New York., Springer-Verlag., 1987.

Rodén L. Structure and Metabolism of Connective Tissue Proteoglycan. In: *The Biochemistry of Glycoproteins and Proteoglycans.* Lennarz WJ. (Ed.)., New York., Plenum., 1980.

Rodén L, Baker JR, Cifonelli JA, Mathews MB. Isolation and Characterization of Connective Tissue Polysaccharides. In: *Methods in Enzymology.* Vol 38. 1972

Rodén L, Baker JR, Helting JR, Schwartz N, Stoolmiller AC, Yamagata S, Yamagata T. *Methods Enzymol* 1972;28:638-76.

Rodén L, Horowitz MI. Structure and Biosynthesis of Connective Tissue Proteoglycans. In: *The Glycoconjugates: Mammalian Glycoproteins, Glycolipids, and Proteoglycans.* Volume II., Horowitz MI, Pigman W., (Eds.)., New York., Academic Press., 1978.

Ronquillo HI, Guay J, Ralph WJ, Tallents RH, Katzberg RW, Murphy W. Tomographic Analysis of Mandibular Condyle Position as Compared to Arthrographic Findings of the Temporomandibular Joint. *J Craniomand Disord* 1988;2(2):59-64.

Rosenberg LC, Choi HU, Poole AR, Lewandowska K, and Culp LA. Biological Roles of Dermatan Sulphate Proteoglycans. In: *Functions of the Proteoglycans.* Ciba Foundation Symposium., Chichester., Wiley., 1986.

Rosenberg L. Chemical Basis for the Histologic Use of Safranin-*O* in the Study of Articular Cartilage. *J Bone Joint Surg.* 1971;53A:69-82.

Rosenberg L, Schubert M. In: *The Immunochemistry and Biochemistry of Connective Tissue.*, Rotstein J. (Ed.)., Vol. 3., Basel., Karger., 1970.

Ross JB. Diagnostic Criteria and Nomenclature for TMJ Arthrography in Sagittal Section. Part I. Derangements. *J Craniomand Disord* 1987;1(3):185-201.

Roughley PJ. Structural Changes in the Proteoglycans of Human Articular Cartilage During Aging. *J Rheumatol* 1987;14:14-5.

Roughley PJ, White RJ. Age-Related Changes in the Proteoglycan Subunits Isolated from Human Articular Cartilage. *J Biol Chem* 1980;255:217.

Ruosiahti E, Yamaguchi Y. Proteoglycans as Modulators of Growth Factor Activities. *Cell* 1991;64:867-9.

Saito H, Yamagata T, Suzuki S. Enzymatic Methods for the Determination of Small Quantities of Isomeric Chondroitin Sulphates. *J Biol Chem* 1968;243:1534-42.

Salisbury JR. Lack of Keratan Sulphate in the Human Notochord. *J Anat* 1988;157:175-9.

Salo L, Raustia A, Pernu H, Virtanen K. Internal Derangements of the Temporomandibular Joint: A Histochemical Study. *J Oral Maxillofac Surg* 1991;49(2):171-6.

Sarnat BG, Laskin DM. *The Temporomandibular Joint: A Biological Basis for Clinical Practice.* 4th Ed., Philadelphia, W.B. Saunders Company, 1992.

Savalle WPM. Some Aspects of the Morphology of the Human Temporomandibular Joint Capsule. *Acta Anat* 1988;131:292-6.

Scapino RP. Histopathology Associated with Malposition of the Human Temporomandibular Joint Disc. *Oral Surg* 1984;58:375.

Schmidt K, MacNair MB. Characterization of the Proteins of Certain Post-Mortem Human Synovial Fluids. *J Clin Invest* 1958;37:708.

Schonherr E, Jarvelainen HT, Sandell LJ, Wight TN. Effects of Platelet Derived Growth Factor and Transforming Growth Factor-Beta 1 on the Synthesis of a Large Versican-Like Chondroitin Sulfate Proteoglycan by Arterial Smooth Muscle Cells. *J Biol Chem* 1991;266(26):17640-7.

Schwartz HC, Kendrick RW. Internal Derangements of the Temporomandibular Joint: Description of Clinical Syndromes. *Oral Surg Oral Med Oral Pathol* 1984;58:24-9.

Scott JE. Histochemistry of Alcian Blue. III. The Molecular Biological Basis of Staining by Alcian Blue 8GX and Analogous Phthalocyanins. *Histochemie* 1972;32(3):191-212.

Scott JE. Proteoglycan-Collagen Interactions. In: *Functions of the Proteoglycans.* Ciba Foundation Symposium., Chichester., Wiley., 1986;124:104.

Scott JE. Proteoglycan-Fibrillar Collagen Interactions. *Biochem J* 1988;252:313-23.

Scott JE. Proteoglycan: Collagen Interactions in Connective Tissues. Ultrastructural, Biochemical, Functional and Evolutionary Aspects. *Int J Biol Macromol* 1991;13(3):157-61.

Scott JE, Dorling J. Differential Staining of Acid Glycosaminoglycans (Mucopolysaccharides) by Alcian Blue in Saly Solutions. *Histochemie* 1965;5:221.

Scott JE, Haigh M. Proteoglycan-type I Collagen Fibril Interactions in Bone and Non-calcifying Connective Tissues. *Bioscience Reports* 1985;5:71-81.

Scott JE, Orford CR, Hughes EW. Proteoglycan-Collagen Arrangements in Developing Rat Tail Tendon. *Biochem J* 1981;195(3):573-81.

Scott JH. *Dento-facial Development and Growth.* Oxford., Pergamon Press., 1967.

Scott PG, Dodd CM. The N-linked Oligosaccharides of Bovine Skin PDS. *Biochem Soc Trans* 1989;17:1031-2.

Scott PG, Dodd CM. Self Aggregation of Bovine Skin PDS Promoted by the Removal of the Three N-linked Oligosaccharides. *Connect Tissue Res* 1990;24:225-36.

Scott PG, Nakano T, Dodd CM, Pringle GA, Kuc IM. Proteoglycans of the Articular Disc of the Bovine Temporomandibular Joint. II. Low Molecular Weight Dermatan Sulfate Proteoglycan. *Matrix* 1989;9(4):284-92.

Scott PG, Winterbottom N, Dodd CM, Edwards E, Pearson CH. A Role for Disulphide Bridges in Protein Core in the Interaction of PDS and Collagen. *Biochem Biophys Res Comm* 1986;138(3):1348-54.

Seno N, Anno K, Yaegashi Y, and Okuyama T. Microheterogeneity of Chondroitin Sulfates from Various Cartilages *Connect Tissue Res* 1975;3:87.

Sether LA, Yu S, Haughton VM, Fischer ME. Intervertebral Disk: Normal Age-Related Changes in MR Signal Intensity. *Radiology* 1990;177(2):385-8.

Shengyi T, Xu Y. Biochemical Properties and Collagen Fiber Orientation of TMJ Discs in Dogs: Part 1. Gross Anatomy and Collagen Fiber Orientation of the Discs. *J Craniomandib Disord Facial Oral Pain* 1991;5(1):28-34.

Shengyi T, Xu Y, Mongshi C, Yongnian L. Biochemical Properties and Collagen Fiber Orientation of Temporomandibular Joint Discs in Dogs: Part 2. Tensile Mechanical Properties of the Discs. *J Craniomandib Disord Facial Oral Pain* 1991;5(2):107-14.

Shepard N, Mitchell N. The Localization of Proteoglycan by Light and Electron Microscopy using Safronin *O*. *Anat Rec* 1977;17(4):463-76.

Shimada E, Kudoh N, Tomioka M, Matsumura G. Separation of Unsaturated Chondroitin Sulfate Disaccharides in Thin-Layer Chromatography on Silica Gel and Their Quantitative Determination by Densitometry. *Chem Pharm Bull* 1987;35(4):1503-8.

Shimizu S, Ito M, Dean J. Glycoproteins of Mouse Zona Pellucida. *Biochem Biophys Res Comm* 1982;109:449-54.

Sicher H. Structure and Functional Basis for Disorders of the Temporomandibular Articulation. *J Oral Surg* 1955;13:275-9.

Sicher H. Growth of the Mandible. *Am J Orthod* 1957;33:30.

Sigvard K. Topographical Distibution of Sulphated Glycosaminoglycans in Human Temporomandibular Joint Disks. *J Oral Pathol* 1976;5:265-76.

Silbert JE. Incorporation of ^{14}C and ^{3}H from Nucleotide Sugars into a Polysaccharide in the Presence of a Cell-Free Preparation from Cartilage. *J Biol Chem* 1964;239:1310.

Silbert JE. Biosynthesis of Mucopolysaccharides and Protein-Polysaccharides. In: *Molecular Pathology of Connective Tissues.* Perez-Tomayo R, Rojkind M. (Eds.)., New York., Marcel Dekker., 1973.

Silbert JE. Biosynthesis of Chondroitin Sulfate. Chain Termination. *J Biol Chem* 1978;253(19):6888-92.

Silbert JE. Structure and Metabolism of Proteoglycans and Glycosaminoglycans. *J Invest Dermat.* 1982;79:31.

Silbert JW, Freilich LS. Biosynthesis of Chondroitin Sulfate by Golgi-Apparatus-Enriched Preparation from Cultures of Mouse Mastocytoma Cells. *Biochem J* 1980;190:307-13.

Silva DG. Further Ultrastructural Studies on the Temporomandibular Joint of the Guinea Pig. *J Ultrastruct Res* 1969;26(1):148-66.

Slater RJ. *Radioisotopes in Biology: A Practical Approach.* Slater RJ. (Ed.)., New York., Oxford University Press., 1990.

Smeele LE. Ontogeny of Relationship of Human Middle Ear and Temporomandibular (Squamomandibular) Joint. *Acta Anat* 1988;131:338-41.

Sokoloff L. *The Joints and Synovial Fluid.* Sokoloff L. (Ed.)., Volume I., New York., Academic Press 1978.

Sokoloff L. *The Joints and Synovial Fluid.* Sokoloff L. (Ed.)., Volume II., New York., Academic Press 1980.

Solberg WK, Woo MW, Houston JB. Prevalence of Mandibular Dysfunction in Young Adults. *J Am Dent Assoc* 1979;98:25-34.

Solberg WK, Clark GT. *Temporomandibular Joint Problems: Biologic Diagnosis and Treatment.* Quintessence Publishing Co., Inc., Chicago., 1980.

Sperber GH. *Craniofacial Embryology Dental Practitioner Handbook.* no. 15, 4th ed. Butterworths 1989:161-4.

Spooner BS, Thompson-Pletscher HA. Matrix Accumulation and the Development of Form: Proteoglycans and Branching Morphogenesis. In: *Regulation of Matrix Accumulation.* Mechan R., (Ed.)., London., Academic Press., 1986;400-40.

Sprinz R. The Role of the Mensiscus in the Healing Process Following Excision of the Articular Surfaces of the Mandibular Joint in Rabbits. *J Anat.* 1963;97:345.

Stanescu V, Chaminade F, Muriel MP. Age-Related Changes in Small Proteoglycans of Low Buoyant Density of Human Articular Cartilage. *Connect Tiss Res* 1988;17(4):239-52.

Stockwell RA. Lipid Content of Human Costal and Articular Cartilage. *Ann Rheum Dis* 1967a;26(4):481-6.

Stockwell RA. The Lipid and Glycogen Content of Rabbit Articular and Non-Articular Hyaline Cartilage. *J Anat* 1967b;102:87.

Stockwell RA. *Biology of Cartilage Cells.* New York., Cambridge University Press., 1979.

Stow JL, Kjellén L, Unger E, Höök M, Farquhar MG. Heparan Sulphate Proteoglycans are Concentrated on the Sinusoidal Plasmalemmal Domain and in Intracellular Organelles of Hepatocytes. *J Cell Biol* 1985;100:975-80.

Strauss F, Christen A, Weber W. The Architecture of the Disk of the Human Temporomandibular Joint. *Helv Odont Acta* 1960;4(1):1-4.

Strider W, Pal S, Rosenberg L. Comparison of Proteoglycans from Bovine Articular Cartilage. *Biochim Biophys Acta* 1975;379(1):271-81.

Sunderland CA, Redman CWG, Stirrat GM. Monoclonal Antibodies to Human Synccytiotrophoblast. *Immunology* 1981;43:541-6.

Sunderland CA, Bulmer JN, Luscombe M, Redman CWG, Stirrat GM. Immunohistological and Biochemical Evidence for a Role for Hyaluronic Acid in the Growth and Development of the Placenta. *J Reproduct Immunol* 1985;8:197-212.

Sweet MBE, Thonar EJMA, Immelman AR. Regional Distribution of Water and Glycosaminoglycan in Immature Articular Cartilage. *Biochem Biophys Acta* 1977;173.

Symons NBB. The Development of the Human Mandibular Joint. *J Anat* 1952;86(3):326-33.

Takenoshita Y. Development with Age of the Human Mandibular Condyle: Histological Study. *J Craniomandib Pract* 1987;5(4):317-23

Tammi R, Tammi M, Hakkinen L, Larjava H. Histochemical Localization of Hyaluronate in Human Oral Epithelium Using a Specific Hyaluronate-Binding Probe. *Archs Oral Biol* 1990;35(3):219-24.

Ten Cate AR. *Oral Histology: Development, Structure, and Function.* 3rd Ed., St. Louis., C.V. Mosby, 1989.

Theocharis DA. Comparisons Between Extracted and Residual Proteoglycans on the Glycosaminoglycan Level and Changes with Aging. *Int J Biochem* 1985;17(2):155-60.

Theocharis DA. Age-Related Changes of Proteoglycans Subunits from Sheep Nasal Cartilage. *Int J Biochem* 1985;17(4):479-84.

Thilander B. The Structure of the Collagen of the Temporomandibular Disc in Man. *Acta Odont Scand* 1964;22:135-49.

Thilander B, Carlsson G, Ingervall B. Postnatal Development of the Human Temporomandibular Joint: I. A Histological Study. *Acta Odont Scand* 1976;34:117-26.

Thomas M, Bronstein SL. *Arthroscopy of the Temporomandibular Joint.* Philadelphia, W.B. Saunders Company, 1991.

Thonar EJMA, Kuettner KE. Biochemical Basis of Age-Related Changes in Proteoglycans. In: *Biology of Proteoglycans.* Wight TN, Mechan RP. (Eds.)., London., Academic Press., 1987; 211-46.

Thonar E, Lohmander L, Kimura J, Fellini S, De Luca S, Nilsson B, Hascall VC. Structure and Biosynthesis of Oligosaccharides on Proteoglycans. In: *Proceedings of the VIth International Symposium on Glycoconjugates.* Yamakawa T, Osawa T, Handa S. (Eds)., Japan Scientific Societies Press., 1981;409-10.

Toller PA. The Synovial Apparatus and Temporomandibular Joint Function. *Br Dent J* 1961;111(10):355-62.

Toller PA. Opaque Arthrography of the TMJ. *Int J Oral Surg.* 1974;3:17.

Tomida M, Koyama H, Ono T. Induction of Hyaluronic Acid Synthetase Activity in Rat Fibroblasts by Medium Charge of Confluent Cultures. *J Cell Physiol* 1975;86:121-30.

Tomida M, Koyama H, Ono T. Effects of Adenosin 3':5'-Cyclic Monophosphate and Serum on Synthesis of Hyaluronic Acid in Confluent Rat Fibroblasts. *Biochem J*

1977;162:539-43.

Toole BP. Glycosaminoglycans in Morphogenesis. In: *Cell Biology of the Extracellular Matrix.* Hay ED. (Ed.)., New York., Plenum., 1981.

Toole BP, Goldberg RL, Chi-Rosso G, Underhill CB, Orkin RY. *The Role of Extracellular Matrix in Development.* Trelstad RL. (Ed.)., New York., Liss., 1984.

Tsiganos CP, Hardingham T, Muir H. Proteoglycans of Cartilage: An assessment of their Structure. *Biochim Biophys Acta* 1971;229(2):529-34.

Tsiganos CP, Muir H. Studies on Protein-Polysaccharides from Pig Laryngeal Cartilage: Extraction and Purification. *Biochem J* 1969;113(5):879-84.

Uldbjerg N, Danielsen CC. A Study of the Interaction *in vitro* Between Type I Collagen and a Small Dermatan Sulphate Proteoglycan. *Biochem J* 1988;251:643-8.

Van der Linden EJ, Burdi AR, de Jongh HJ. Critical Periods in the Prenatal Morphogenesis of the Human Lateral Pterygoid Muscle, the Mandibular Condyle, the Articular disk, and Medial Articular Capsule. *Am J Orthod Dentofac Orthop* 1987;91(1):22-8.

Van Dongen GK. The Formation of the Articular Disc of the Temporomandibular Joint. *Neth Dent J* 1970;77(5):14-22.

Vasan NS, Gutteling EW, Lee CK, Cibischino M, Parsons JR. A Preliminary Study of Mechanically Stress-Induced Changes in the Extracellular Matrix of the Canine Intervertebral Disc. *Spine* 1991;16(3):317-20.

Vilim V, Krajickova J. Proteoglycans of Human Articular Cartilage. *Biochem J* 1991;273:579-85.

Vilim V, Krajickova J. Changes in Proteoglycans in Human Articular Cartilage in Relation to Age. *Cas Lek Cesk* 1990;129(49):1557-61.

Vogel KG, Heinegärd D. Characterization of Proteoglycans from Adult Bovine Tendon. *J Biol Chem* 1985;260(16):9298-306.

Vogel KG, Evanko SP. Proteoglycans of Fetal Bovine Tendon. *J Biol Chem* 1987;262(28):13607-13.

Vogel KG, Paulsson M, Heinegärd D. Specific Inhibition of Type I and Type II

Collagen Fibrillogenesis by the Small Proteoglycan of Tendon. *Biochem J* 1984;223(3):587-97.

Vogel KG, Trotter JA. The Effect of Proteoglycans on the Morphology of Collagen Fibrils Formed *in Vitro*. *Collagen Rel Res* 1987;7:105-14.

Waechter CJ, Lennarz WJ. The Role of Polyprenol-Linked Sugars in Glycoprotein Synthesis. *Annu Rev Biochem* 1976;45:95-112.

Webber C, Glant TT, Roughley PJ, Poole AR. The Identification of Two Populations of Aggregating Proteoglycans of High Buoyant Density Isolated From Post-Natal Human Articular Cartilages of Different Ages. *Biochem J* 1987;248(3):735-40.

Weinberg LA, Chastain JK. New TMJ Clinical Data and the Implication on Diagnosis and Treatment. *J Am Dent Assoc* 1990;120(3):305-11.

Weiss C. Basic Structures of Diarthroidal Joints. In: *Arthroscopic Surgery.*, Parisien JS. (Ed.)., New York., McGraw-Hill., 1988.

Westesson PL. Double Contrast Arthrography and Internal Derangement of the Temporomandibular Joint. *Swed Dent J* 1982; Supplement 13.

Westesson PL. Clinical and Arthrographic Findings in Patients with TMJ Disorders. In: *Diagnosis of Internal Derangements of the Temporomandibular Joint.* Vol. 1., Moffett BC. (Ed.)., Seattle., University of Washington., 1984.

Westesson PL. Structural Hard Tissue Changes in Temporomandibular Joints with Internal Derangements. *Oral Surg Oral Med Oral Pathol.* 1985;59(2):220-4.

Westesson PL, Bronstein SL, Liedberg J. Internal Derangement of the Temporomandibular Joint: Morphologic Description with Correlation to Joint Function. *Oral Surg Oral Med Oral Pathol.* 1985;59(4):323-31.

Westesson PL, Katzberg RW, Lunn RL. *Imaging of the Temporomandibular Joint.* Vol.1., Lunn RL (Ed.)., Baltimore, MD., Williams and Wilkins., 1991.

Wilk AL, King CTG, Pratt RM. Chlorcyclizine Induction of Cleft Palate in the Rat: Degradation of Palatal Gylcosaminoglycans. *Teratol* 1978;18:199-210

Willen MD, Sorrell JM, Lekan CC, Davis BR, Caplan AI. Patterns of Glycosaminoglycan/Proteoglycan Immunostaining in Human Skin During Aging. *J Invest Dermatol* 1991;96(6):968-74.

Williams G, Jackson DS. Two Organic Fixatives for Acid Mucopolysaccharides. *Stain Technol* 1956;31:189-91.

Williams PL, Warwick R. *Gray's Anatomy.* 36th Ed., Edinburgh, Churchill Livingstone, 1980.

Winnemoller M, Schmidt G, Kresse H. Influence of Decorin on Fibroblast Adhesion to Fibronectin. *Eur J Cell Biol* 1991;54(1):10-7.

Wong GB, Weinberg S, Symington JM. Morphology of the Developing Articular Disc of the Human Temporomandibular Joint. *J Oral Maxillafac Surg* 1985;43:565-9.

Wight TM, Mecham RP. *Biology of Proteoglycans.* Wight TM, Mecham RP. (Eds.)., San Diego, CA., Academic Press., 1987.

Wright DM, Moffett BC. The Postnatal Human Temporomandibular Joint. *Am J Anat* 1974;141:235-49.

Wright GC, Wei XQ, McDevitt CA, Lane BP, Sokoloff L. Stimulation of Matrix Formation in Rabbit Chondrocyte Cultures by Ascorbate. 1. Effect of Ascorbate Analogs and Beta-aminopropionitrile. *J Orthop Res* 1988;6(3):397-407.

Yamaguchi Y, Mann DM, Ruoslahti E. Negative Regulation of Transforming Growth Factor-Beta by the Proteoglycan Decorin. *Nature* 1990;346(6281):218-24.

Yamaguchi Y, Ruoslahti E. Expression of Human Proteoglycan in Chinese Hamster Ovary Cells Inhibits Cell Proliferation. *Nature* 1988;336(6196):244-6.

Yoneda M, Yamagata M, Suzuki S, Kimata K. Hyaluronic Acid Modulates Proliferation of Mouse Dermal Fibroblasts in Culture. *J Cell Sci* 1988;90(2):265-73.

Yuodelis RA. The Morphogenesis of the Human Temporomandibular Joint and Its Associated Structures. *J Dent Res* 1966;45(1): 182-91.

Yuodelis RA. Ossification of the Human Temporomandibular Joint. *J Dent Res* 1966;45(1):192-8.

Zenker W. Das Retroarticulare Plastische Polster des Kiefergelenkes und Seine Mechanische Bedeutung. *Z Anat Entwicklungsgesch* 1956;119;375-88.

Zimmerman DR, Ruoslahti E. Multiple Domains of the Large Fibroblast Proteoglycan, Versican *EMBO J* 1989;8(10):2975-81.

Zimmerman HI. The Normal Growth and Remodelling of the Temporomandibular Joint of *Macaca mulatta. Master's Thesis*, University of Washington., Seattle., 1971.